

**SYNTHESIS OF POTENTIAL SH2 DOMAIN LIGANDS CONTAINING PHOSPHATE
GROUP MIMICS**

by

Nathaniel Forrest Ware

BA Chemistry, San Jose State University, 2006

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This thesis was presented

by

Nathaniel Forrest Ware

It was defended on

August 19, 2011

and approved by

Professor Dennis Curran, Department of Chemistry

Professor Kazunori Koide, Department of Chemistry

Thesis Director: Professor Peter Wipf, Department of Chemistry

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Nathaniel Forrest Ware, M.S.

University of Pittsburgh, 2011

This thesis describes the synthesis of two peptidomimetics that contain heterocyclic phosphate group mimics. These compounds are based on a potent STAT3 inhibitor and therefore have the potential of being SH2 domain ligands. Due to problems with the purification of the final products, the mimetic containing the isothiazolidinone (IZD) moiety was obtained as the major component of a mixture in < 90% purity and the 5,6-dihydro-2H-1,2,6-thiadiazine 1,1-dioxide (TDA) analog was isolated as the major component of a mixture in < 90% purity based on NMR analyses.

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ABBREVIATIONS

Ac	acetyl
Ala	alanine
Ar	aryl
Bn	benzyl
Boc	<i>tert</i> -butyloxycarbonyl
BRSM	based on recovered starting material
Bu	butyl
Cbz	carbobenzyloxy
CDK	cyclin-dependent kinases
DB ₅₀	half maximal amount of substrate bound to DNA
DIPEA	diisopropylethylamine
DMAP	4- <i>N,N</i> -dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dppf	1,1'-bis(diphenylphosphino)ferrocene
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
equiv	equivalents

Et	ethyl
F ₂ Pmp	α,α -difluorophosphinomethylphenylalanine
Fmoc	9-fluorenylmethoxycarbonyl
Gln	glutamine
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
Het	heterocycle
HOBt	hydroxybenzotriazole
IBCF	isobutyl chloroformate
IC ₅₀	half maximal inhibitory concentration
IFN	interferon
IL	interleukin
IZD	isothiazolidinone
JAK	Janus kinase
<i>L</i>	levorotatory
LCMS	liquid chromatography/mass spectrometry
Leu	leucine
Lys	lysine
mCPBA	<i>meta</i> -chloroperoxybenzoic acid
Me	methyl
NMM	<i>N</i> -methyl morpholine
<i>o</i> -DCB	<i>ortho</i> -dichlorobenzene
P	protecting group

Ph	phenyl
Phe	phenylalanine
PNPP	<i>para</i> -nitrophenylphosphate
Pro	proline
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
pTyr	phosphotyrosine
Ser	serine
SH2	Src homology 2
STAT	signal transducer and activator of transcription
Su	succinimide
<i>t</i>	tertiary
TAD	transcriptional activation domain
T3P	1-propylphosphonic acid cyclic anhydride
TBAB	tetra- <i>n</i> -butylammonium bromide
TDA	5,6-dihydro-2H-1,2,6-thiadiazine 1,1-dioxide
<i>tert</i>	tertiary
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
Trt	trityl
Tyr	tyrosine

μW

microwave irradiation

1.0 INTRODUCTION AND BACKGROUND INFORMATION

1.1 PHOSPHATE GROUP MIMICS

Due to the activity of phosphorylated proteins in nature and the poor bioavailability of phosphorylated pharmaceutical compounds, many phosphate group mimics have been developed for the ubiquitous phosphotyrosine moiety.¹⁻³ One strategy towards creating these mimics has been to stabilize the compound against the phosphatases present in cells. To accomplish this, the oxygen linker present between the phosphate and the aromatic ring was replaced with a methylene group to create the phosphinomethylphenylalanine (Pmp) moiety (Figure 1-1a).⁴ This small change has been shown to increase the stability of the phosphate group towards enzymatic cleavage, but it also reduced binding ability towards the desired targets.^{2,5} This has been rationalized to be a consequence of an increase in the pKa of the phosphonate group at physiological pH from 5.7 for the phosphotyrosyl group to a value of 7.1 for the Pmp group.⁵ This change in pH results in a reduction of the formal negative charge of -2 for the fully deprotonated phosphotyrosyl group versus a -1.5 for the Pmp group that is in equilibrium between its mono-protonated and unprotonated states. To remedy this problem, the methylene linker was fluorinated to create the F₂Pmp phosphotyrosine mimic (Figure 1-1a), which restored the binding affinity to the levels of the parent compound.⁶⁻⁸

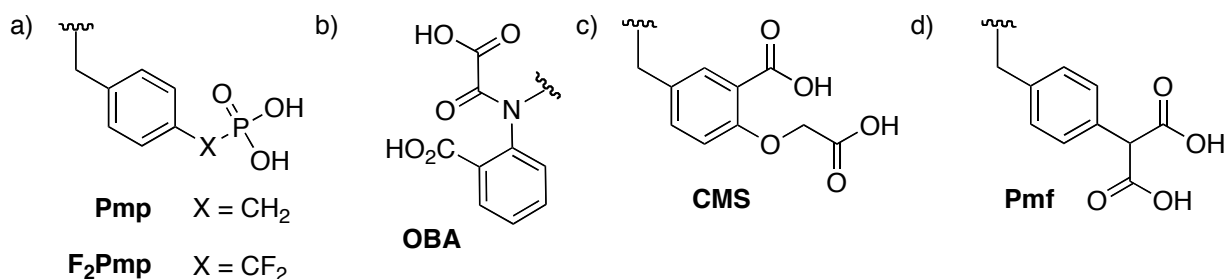


Figure 1-1. Common phosphotyrosine mimics.

While these groups have been resistant to enzymatic cleavage, they still have poor membrane transport properties.^{2,3,9} Switching from a phosphate-based mimic to a carboxylate group has led to the 2-(oxalylamino)-benzoic acid (OBA) (Figure 1-1b),¹⁰⁻¹³ 3-carboxy-4-carboxymethoxyphenylalanine (CMS) (Figure 1-1c),¹⁴⁻¹⁶ and *p*-malonylphenylalanine (Pmf) (Figure 1-1d)^{17,18} groups. These groups have better membrane permeability and have been used successfully in high affinity binding molecules.

An alternate approach to making a cell permeable inhibitor is to use compounds with a high number of hydrogen bond acceptors while keeping the molecule either neutral or mono-ionic at physiological pH. This strategy allows for the strong electrostatic element of the dianionic phosphate group to be mimicked while still allowing the inhibitor to pass through the cell membranes. Researchers at AstraZeneca published that thiadiazolidinone **1-1** is an inhibitor of protein tyrosine phosphatase-1B (PTP1B) (Figure 1-2).¹⁹ This compound had an IC₅₀ value of 2.47 μM. A tetronic acid moiety has also been used as a phosphate mimic in compound **1-2** to inhibit the VH-1 related (VHR) and Cdc25B protein phosphatases with IC₅₀ values of 11.6 μM and 2.2 μM, respectively.²⁰ Sugen, Inc. utilized the trifluoromethyl sulfonyl group in ether **1-3**

as a phosphate mimic to inhibit a range of PTPs in the μM range and thus obtained one of the first uncharged mimics to show substrate-competitive inhibition.²¹

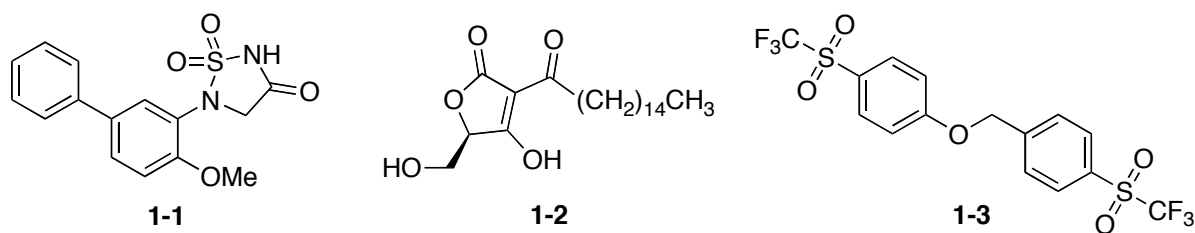
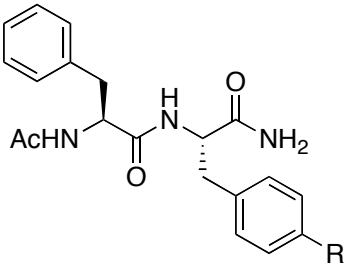
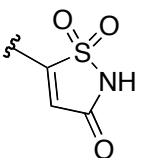
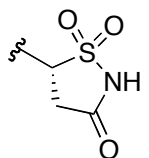
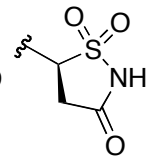
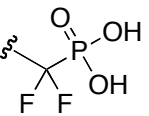


Figure 1-2. Miscellaneous phosphate group mimics.

The Incyte Corporation developed isothiazolidinone (IZD) as a phosphate mimic using a structure-based design and then incorporated it into a series of PTP1B inhibitors (Table 1-1).²² The unsaturated **1-4**, along with (*S*)-IZD **1-5** was active against PTP1B with IC_{50} values of 3000 nM and 190 nM, respectively. The (*R*)- derivative (**1-6**) was found to be more than five times less active than the unsaturated IZD **1-4**, and more than eighty times less active than its epimer. The (*S*)-IZD (**1-5**) was also found to be ten times more potent than the corresponding inhibitor containing the F_2Pmp peptide mimic (**1-7**).

Table 1-1. Comparison of the IZD stereoisomers.



entry	compound	R	PTPIB ^a IC ₅₀ (nM)	entry	compound	R	PTPIB ^a IC ₅₀ (nM)		
1	1-4	IZD		3000	3	1-6	(<i>R</i>)-IZD		16000
2	1-5	(<i>S</i>)-IZD		190	4	1-7	F ₂ Pmp		1750

^a PNPP enzyme assay.

Shortly after publishing the initial IZD paper, the Incyte Corporation published non-peptidic inhibitors **1-8** and **1-9** (Figure 1-3).^{23,24} These compounds were shown to have IC₅₀ values of 35 nM and 480 nM, respectively, against PTP1B, but, more importantly, were found to be cell permeable in the caco-2 adenocarcinoma cell line.²⁴

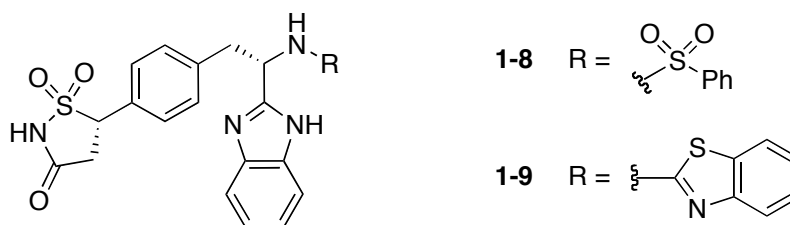
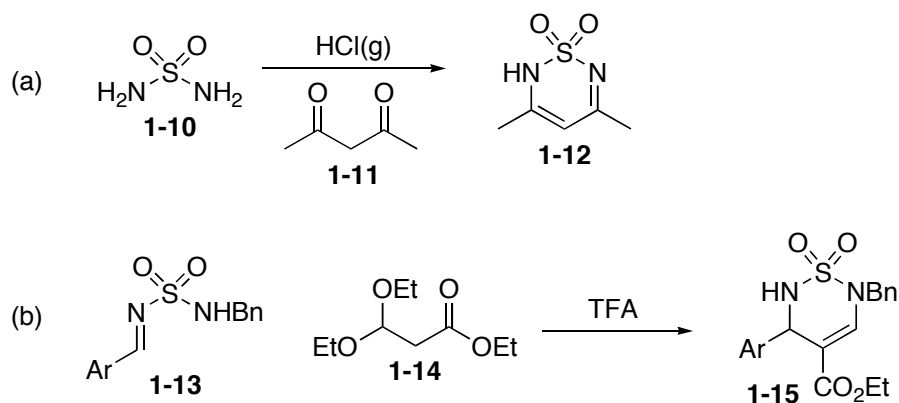


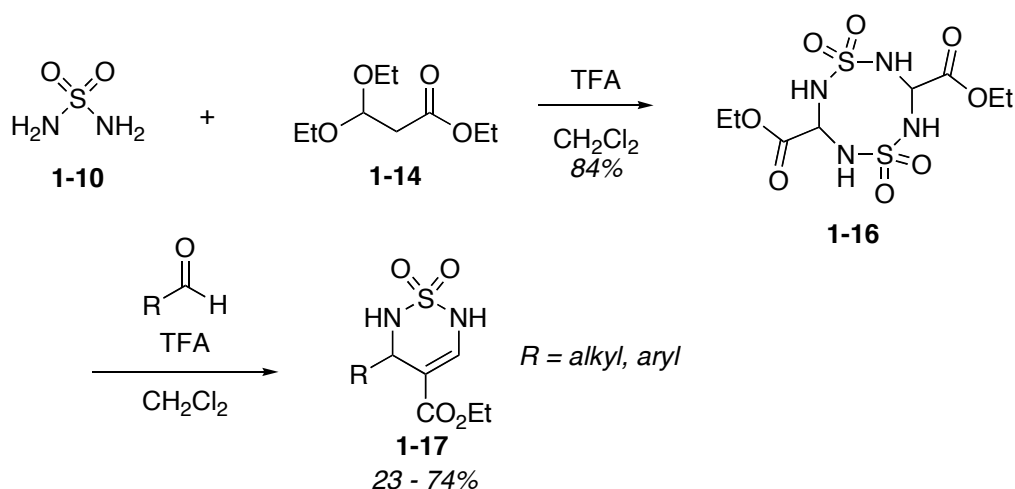
Figure 1-3. Cell permeable IZD containing PTP1B inhibitors.

The structurally analogous 5,6-dihydro-2H-1,2,6-thiadiazine 1,1-dioxide (TDA) moiety was targeted by the Wipf group as phosphate group mimic based on other biologically active compounds that contain the sulfamide group.^{19,25,26} Prior to our work, the procedures to synthesize this moiety involved either the condensation of sulfamide with a 1,3-dicarbonyl compound (Scheme 1-1, equation a) or the condensation of an arylidenesulfamide and ethyl 3,3-diethoxypropionate (Scheme 1-1, equation b).²⁷⁻³¹



Scheme 1-1. Synthetic strategies towards the thiadiazine moiety.

Our initial work found that the processes outlined in Scheme 1-1 were unreliable (unpublished data). During optimization studies, it was found that sulfamide (**1-10**) and ethyl 3,3-diethoxypropionate (**1-14**) could be condensed to form the eight-membered dimer **1-16** in good yields (Scheme 1-2). A suspension of dimer **1-16** in CH₂Cl₂ could then be condensed with a variety of aldehydes using ten equivalents of TFA to provide compounds of type **1-17** with improved reaction times and with yields ranging from 23–74 %.



Scheme 1-2. Wipf group strategy for constructing the thiadiazine moiety.

The TDA moiety has four points that allow for diversification (Figure 1-4a). The R₁ position can be varied by condensing different aldehydes with dimer **1-16**. Both alkyl and aryl substituents have been included into this position.

Initial attempts at selective N-alkylation using standard S_N2 conditions provided mixtures of addition at both R₂ and R₃ sites. Switching to Mitsunobu reaction conditions allowed for the

selective alkylation at R₂ (Figure 1-4b).³² This allows for the incorporation of alkyl, benzyl, allylic and propargylic side chains.

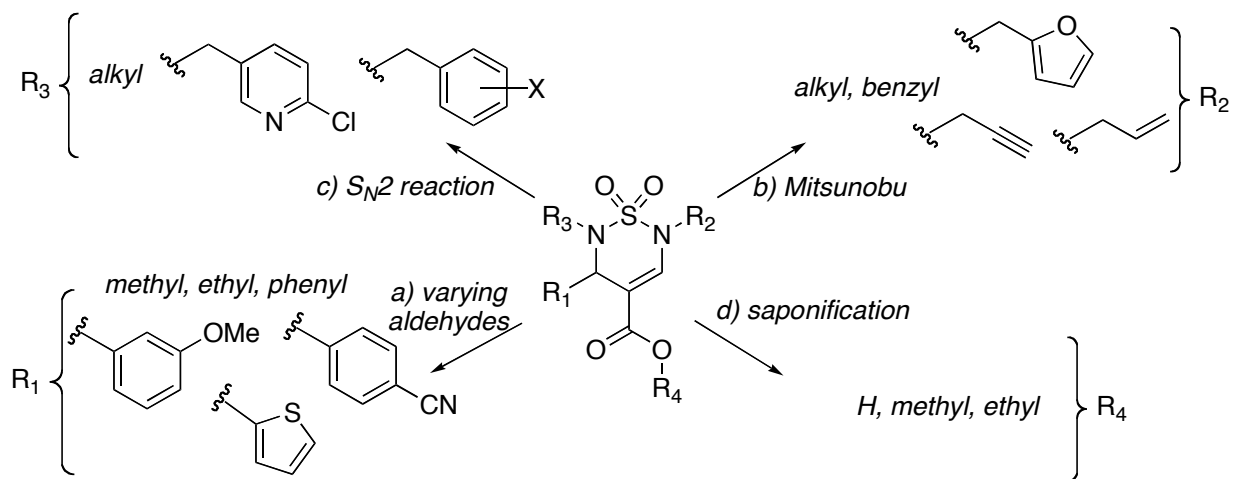
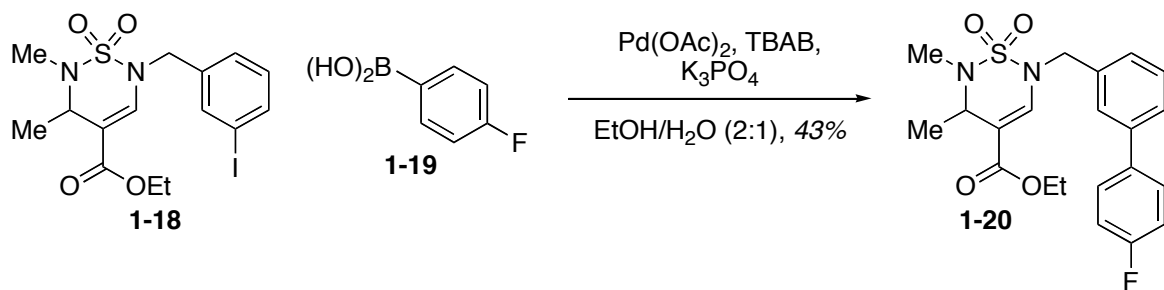


Figure 1-4. Points of elaboration of the TDA moiety.

Once the R₂ position has been occupied, the R₃ position can be readily alkylated (Figure 1-4c).³² This has allowed for the incorporation of alkyl, benzylic and side chains containing pyridine rings. Varying amounts of halogenation on the benzyl rings is well tolerated.

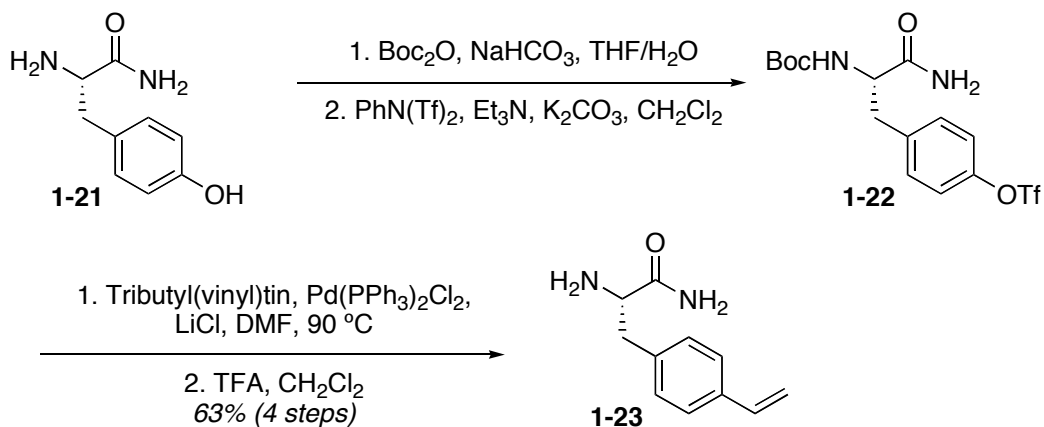
The R₄ position has been saponified to the acid (Figure 1-4d).³³ This position could be amenable towards further functionalization through Curtius rearrangements, decarboxylation, or amidation and current efforts in the group are focused on the realization of these transformations.

The TDA group has also been shown to be stable towards palladium catalyzed cross coupling (Scheme 1-3).³⁴ TDA derivative **1-18** was coupled with boronic acid **1-19** to afford diaryl **1-20** in 43% yield. These elaborations allow for the possibility of the TDA moiety to be incorporated into many different types of scaffolds.



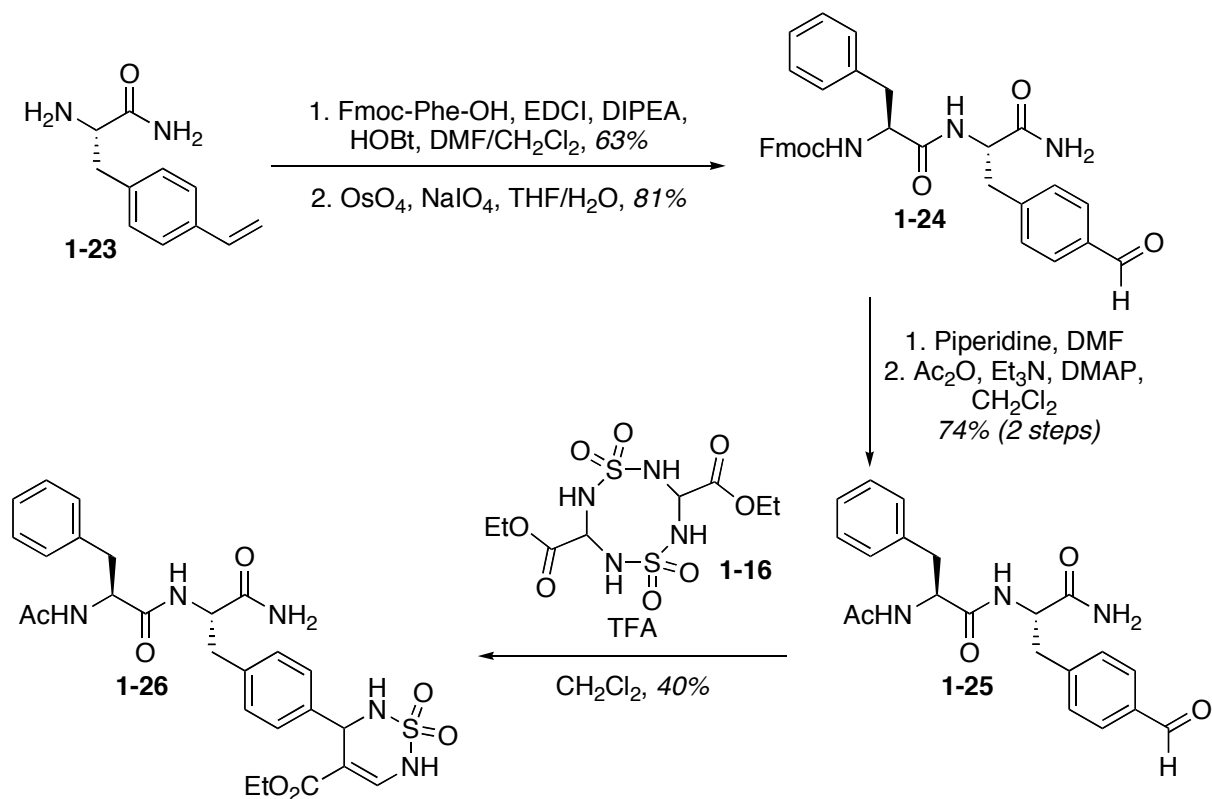
Scheme 1-3. Suzuki coupling with TDA coupling partner.

As a proof of concept, a dipeptide corresponding to the series of PTP1B inhibitors synthesized in the development of the IZD group (**1-4** through **1-7**) was prepared.^{22,35} Boc protection of *L*-tyrosinamide followed by triflation of the phenol afforded **1-22** (Scheme 1-4). Stille coupling with tributyl(vinyl)tin and subsequent Boc deprotection gave vinyl derivative **1-23** in 63% over four steps.



Scheme 1-4. Synthesis of vinyl derivative **1-23**.

Peptide coupling with Fmoc protected phenylalanine followed by Johnson-Lemieux cleavage of the olefin afforded aldehyde **1-24** (Scheme 1-5). A protecting group switch to the acetyl group gave **1-25**, which was then treated with **1-16** to give dipeptide **1-26** in 40% yield. The peptide was found to be active against MKP-1 and MKP-3 phosphatases.



Scheme 1-5. Formation of dipeptide **1-26**.

1.2 SH2 OVERVIEW

Src homology 2 (SH2) regions were originally discovered in the Src family of protein-tyrosine kinases (PTK) and have subsequently been found to be present in 110 separate proteins.³⁶ These regions are approximately 100 amino acids long, highly conserved domains involved in cellular signal transduction.³⁷⁻⁴⁰ They specifically bind to peptide sequences containing a phosphotyrosine (pTyr) moiety and, along with PTK and protein-tyrosine phosphatases (PTP), provide the framework for cell signaling.^{39,41} The peptide complex formed from the coordination between the phosphorylated protein and its corresponding SH2 domain containing ligand acts to modulate a variety of cellular processes including altering gene expression, regulating kinase activity, and inducing ubiquitination.⁴²⁻⁴⁴ Not surprisingly, this requires a high level of specificity between the phosphorylated sequence and its corresponding SH2 domain partner to access the correct cellular function. SH2 domains have been found to be very sensitive towards subtle changes in the residues immediately following the pTyr group and examination of the binding affinity of many short phosphopeptide sequences across a range of SH2 domains has been studied.^{45,46}

1.3 STAT3: MECHANISM OF ACTION AND INHIBITION

The mammalian STAT protein family consists of seven members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6) and share six conserved structural domains.⁴⁷⁻⁵⁰ These domains are the amino terminal domain, the coiled-coil domain, the DNA binding domain, a linker domain, the SH2 domain, and a transcriptional activation domain (Figure 1-5).

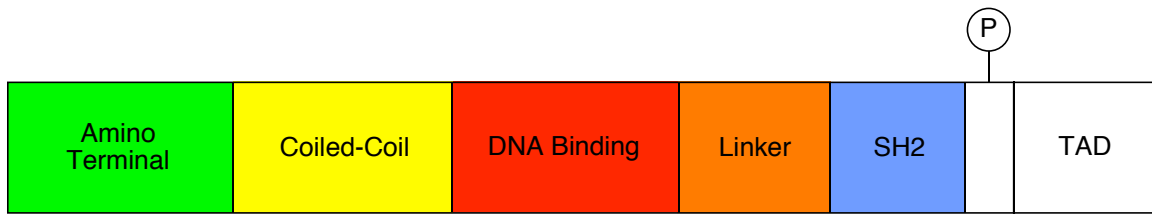


Figure 1-5. The conserved domains of the STAT family of proteins.

The transcriptional activation domain is the most divergent of the domains and is the main contributor to the specificity of the individual STATs.⁵¹⁻⁵⁶ A smaller set of STAT proteins are also present in primitive eukaryotes and each of those STAT proteins are tied to multiple signaling pathways.⁵⁷⁻⁵⁹ This points to STATs 3 and 5 being the first mammalian STAT proteins to evolve as they show the greatest pleiotropy of the family in humans.

STATs 1 and 2 can form homo and heterodimers and predominantly transduce signals for type I and type II interferons (IFN).^{60,61} Knockout studies have shown that STAT1 is important in IFN-dependant immune responses in both viral and microbial agents. Using knockout studies with STAT2-deficient mice has implicated it in the type I IFN signaling pathway.⁶² STAT2 is the only STAT not known to bind to growth arrest-specific (GAS) genes following its activation.

The expression of STAT4 is limited to NK cells, dendritic cells, and T lymphocytes. STAT4 was shown to be activated by IL-12, which plays an essential role in the development of Th1 subset of T cells.^{63,64} Knockout studies confirmed this connection.^{65,66}

STATs 5a and 5b are encoded by linked genes and are expressed in all tissue types.⁶⁷ They are activated by a host of proteins including the IL-2 and IL-3 families, growth hormone, prolactin and EPO and TPO.^{68,69} Knockout studies show that despite sharing 96% of their

sequence, STATs 5a and 5b show distinct phenotypes.^{50,67} STAT5a knockout mice showed the PRL dependant mammary gland to be defective in its development.^{70,71} The STAT5b knockout mice showed traits similar to growth hormone receptor deficient mice.^{71,72} As high levels of growth hormone found in males activate STAT5b, the deletion of this protein affected the males more than the females. The male knockout mice had much more female physical characteristics and an altered expression of gender specific genes to favor the female variant. It is believed that each of the STAT5 proteins can partially substitute for the other as double knockout mice have the combined phenotypes of the individual knockouts, only more severe in each.⁷²

STAT6 knockout mice have been found to be defective in their ability to produce the Th2 subset of helper T cells.⁷³⁻⁷⁵ It also has been found to be a part of the IL-4 signaling pathway as tissues from STAT6 knockout mice were stimulated with IL-4, the tissue failed to upregulate the expression of the MHC class II, IL-4R, and low affinity IgE receptors.

The STAT3 protein contributes to many different cellular processes including promoting cell growth, maintaining an undifferentiated state of embryonic stem cells, inhibiting IL-6 induced neuronal differentiation in PC12 cells, and mediating cytokine induced terminal differentiation in a variety of cell types.⁷⁶⁻⁸⁴ Constitutive STAT3 activity has also been implicated in a wide range of cancers and has made targeting this protein attractive for cancer therapies.⁸⁵⁻⁸⁹

The overall STAT signaling cascade is very similar for all of the proteins of the family. It begins with an extracellular ligand binding to a transmembrane protein that, in turn, activates the intercellular Janus kinase (JAK) (Figure 1-5).^{90,91} The JAK protein then phosphorylates in the SH2 domain of STAT, which instigates dissociation from the JAK protein complex.^{92,93} The phosphorylated monomer then undergoes dimerization through reciprocal phosphotyrosine-SH2

interactions.^{42,47,48,94} The dimer then binds to the either transport protein importin $\alpha 5$ or $\alpha 7$ and translocates the complex to the nucleus where it attaches to the DNA and begins transcription.⁹⁵⁻⁹⁷ The only gene that a STAT protein is known to directly transcribe is VEGF but it they are believed to activate the BCL-X_L, MCL1, survivin, MYC, and the cyclin D1 and D2 genes.⁹⁸⁻¹⁰⁹ At an unknown point during this pathway, a serine residue is also phosphorylated and it is believed that it assists in the binding of the STAT dimer to DNA.^{55,110-113}

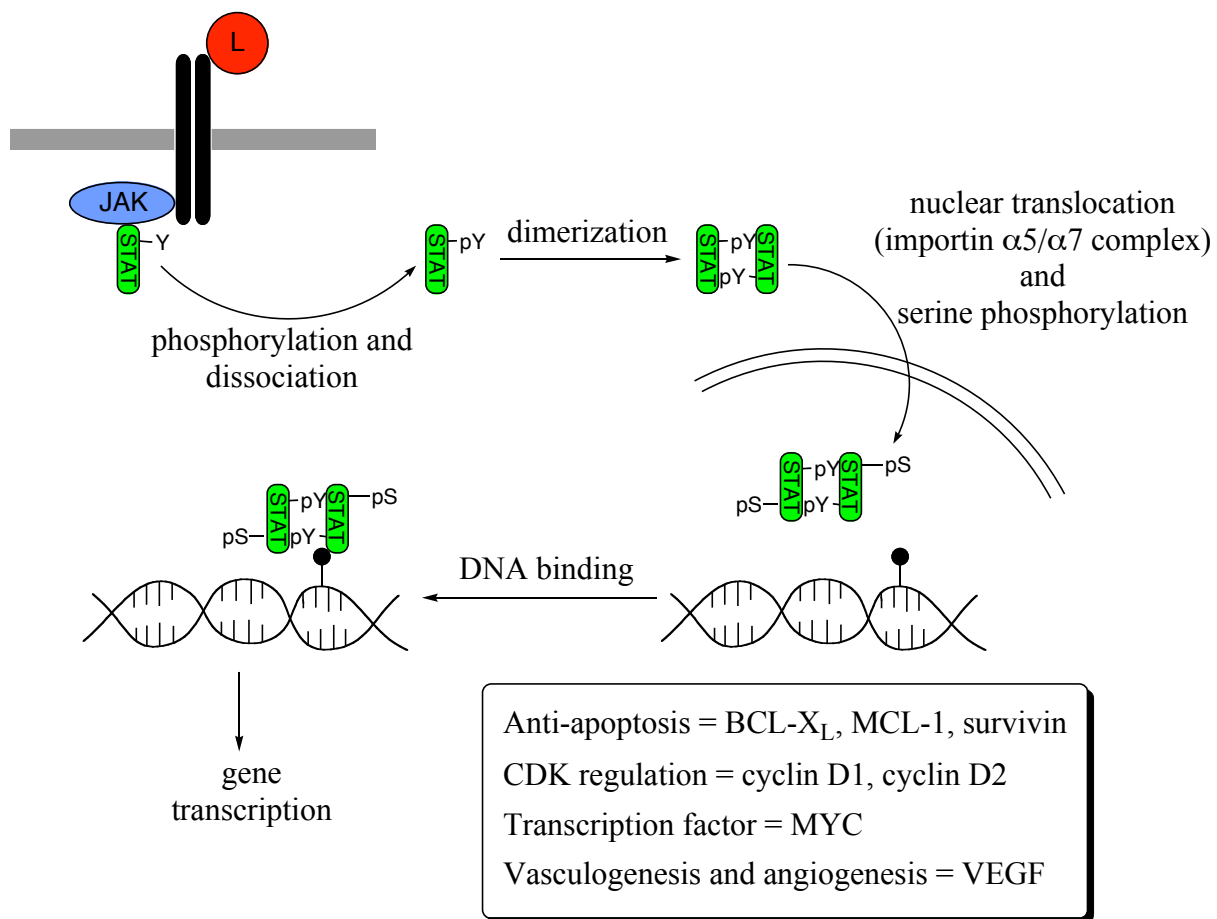


Figure 1-6. General STAT protein cellular mechanism.

Targeting the SH2 domain of STAT3 to disrupt dimerization to block the mechanism prior to the translocation to the nucleus has been described as one of the major strategies towards STAT inhibition.⁸⁹ It has been shown that dimerization of the protein is required for nuclear translocation and DNA binding.^{47,48,92,93,95} It is also known that mutations on the protein designed to prevent dimerization halt gene transcription associated with that particular STAT protein.⁹⁵

STAT3 activity can be disrupted using SH2 domain-binding peptide **1-27** (Figure 1-6).¹¹⁴ This hexamer was found to inhibit DNA binding with a DB₅₀ value of 235 μ M. Scanning for the essential amino acid elements in the peptide revealed two strongly binding tripeptides: Pro-pTyr-Leu (**1-28**) and Ala-pTyr-Leu (**1-29**) with DB₅₀ values of 182 μ M and 217 μ M, respectively. All three of these active peptide sequences were rendered inactive when the phosphate group was removed from the molecule. These sequences were also found to inhibit STAT3 selectively over STAT1 and STAT5.

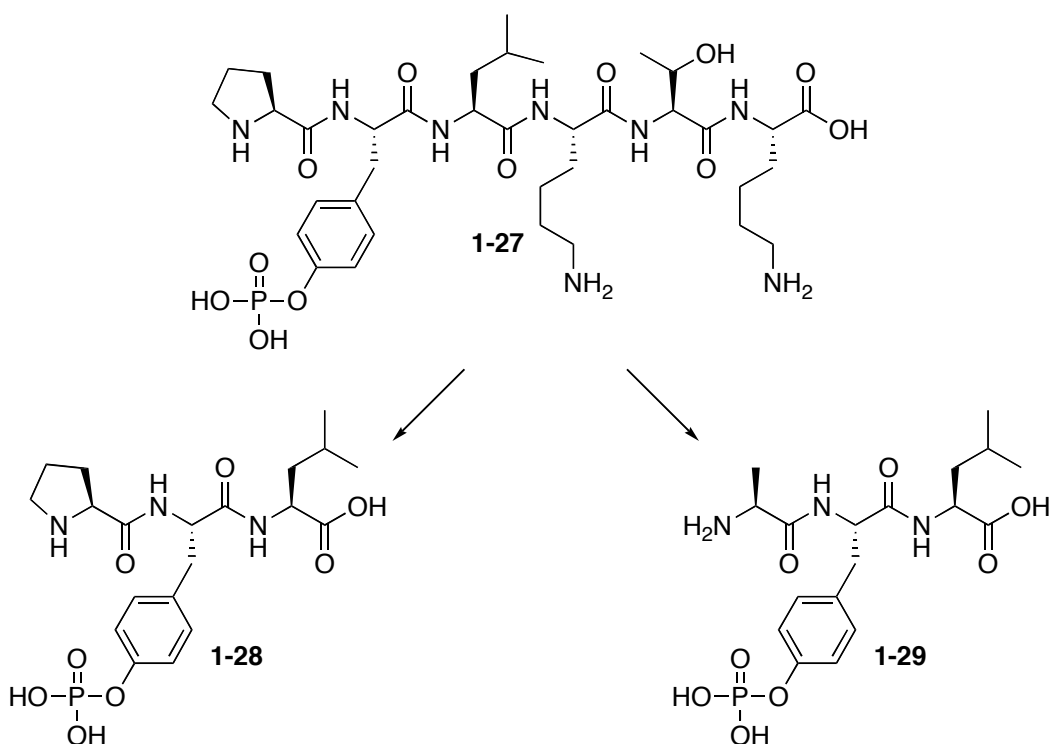


Figure 1-7. Potent STAT3 DNA binding inhibitor **1-27** and the high affinity binding tripeptides derived from it.

It was known early in the history of the STAT proteins that the cytoplasmic domain of the protein gp130 was able to bind to the SH2 region of STAT3.¹¹⁵ Scanning of the gp130 domain showed that it bound to the SH2 region with a pTyr-Xxx-Xxx-Gln motif. This type of “two prongs – two hole” motif is general for binding to the SH2 domains in a variety of proteins.^{46,87} In 2003, gp130 was used as a template to derive peptide (**1-30**), which had an IC₅₀ value of 150 nM against STAT3 binding to DNA (Figure 1-7).¹¹⁶

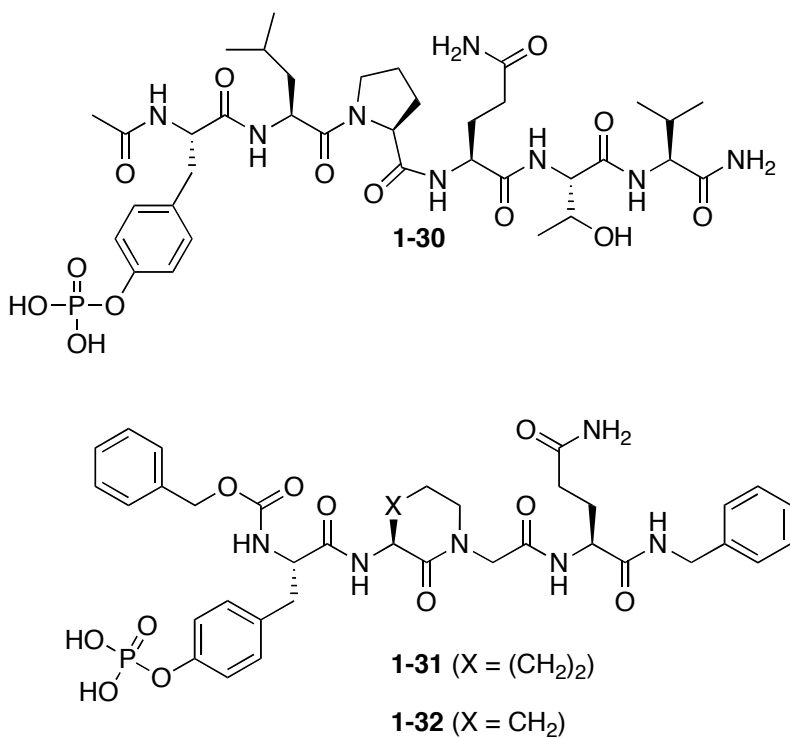


Figure 1-8. Highly potent STAT3 inhibitor **1-30** derived from gp130 and the peptidomimetics **1-31** and **1-32**.

Further derivatization of peptide **1-30** led to the discovery of compound **1-31**, which was found to bind to STAT3 with a $K_i = 190$ nM (Figure 1-7).¹¹⁷ The key component to this compound appears to be the Freidinger lactam segment since reducing the ring size from a seven-membered ring to a six-membered ring (**1-32**) caused the potency to drop significantly to $K_i = 3.13$ μ M.¹¹⁸

2.0 SYNTHESIS OF TWO POTENTIAL SH2 DOMAIN LIGANDS CONTAINING PHOSPHATE GROUP MIMICS

2.1 TARGET SELECTION

Due to the nanomolar activity of **1-31** and the general low bioavailability of phosphorylated derivatives, we undertook the task to replace the phosphate group with mimics that, while still polar, are not strongly ionic and would increase the activity of the compound along with raising its bioavailability. With the choice of both IZD and TDA phosphotyrosine replacements, our target molecules became compounds **2-1** and **2-2**, respectively (Figure 2-1).

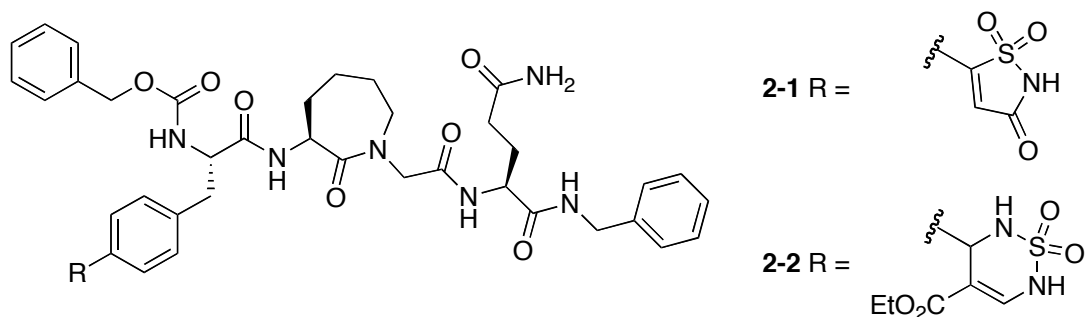
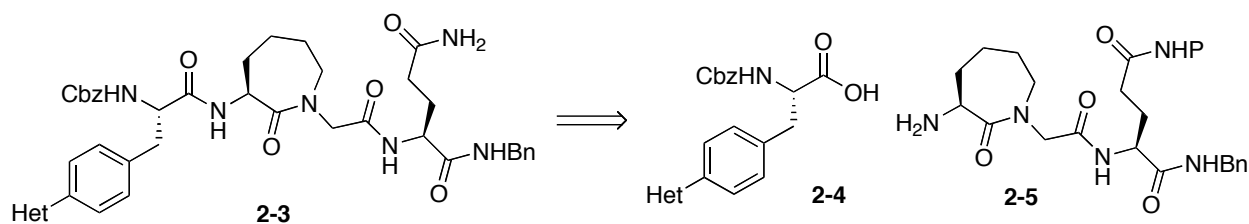


Figure 2-1. Peptidomimetic targets.

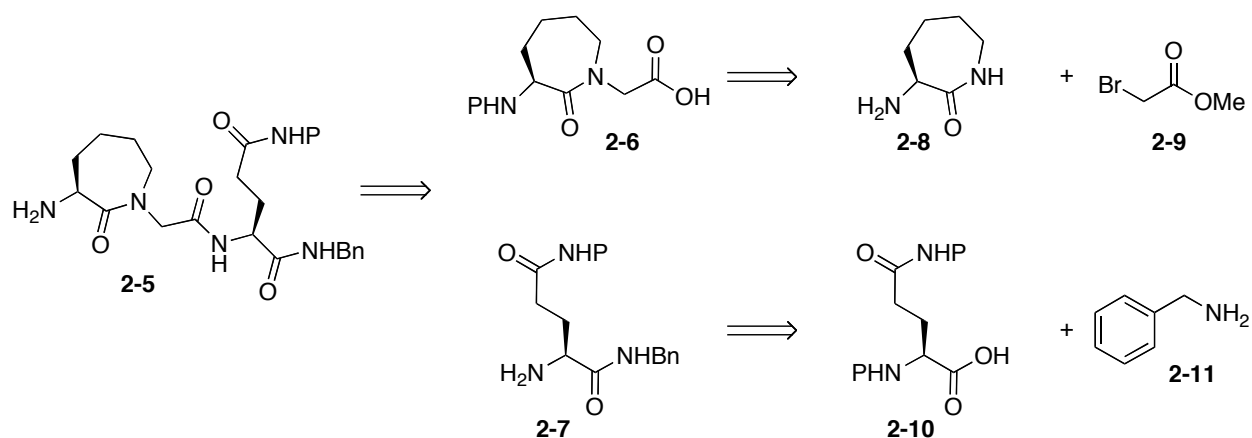
2.2 RETROSYNTHETIC ANALYSIS

The retrosynthetic analysis of target **2-3** began with cleaving the amide bonds to reveal the amino acid subunits. Since the two targets are differentiated by the substitution on the tyrosine residue, it would be desirable for that to be the last bond to form. This would reveal the modified tyrosine acid **2-4** and Freidinger lactam **2-5** as coupling partners (Scheme 2-1).



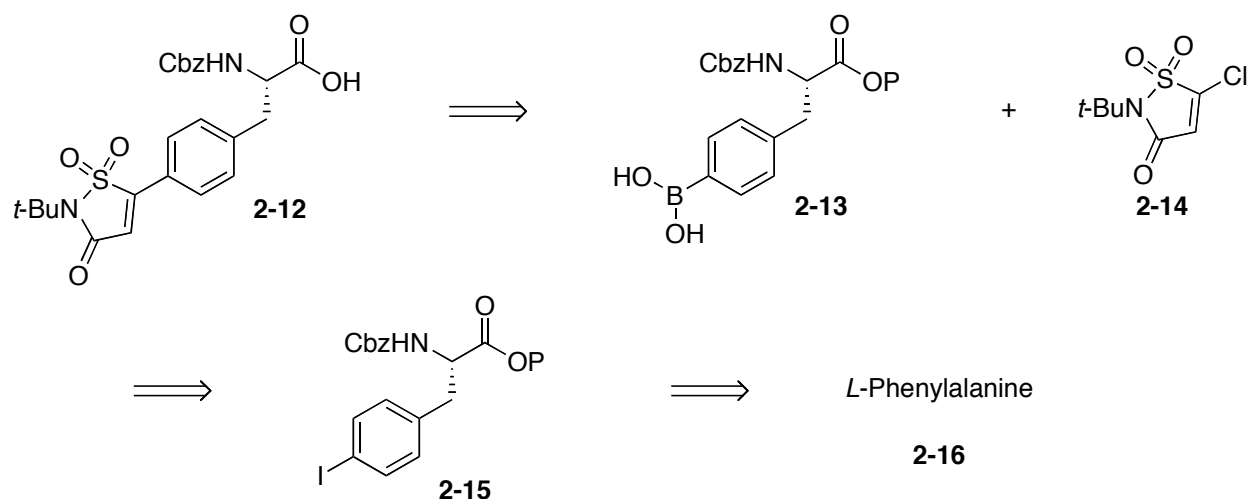
Scheme 2-1. Retrosynthetic analysis of initial bond disconnection.

The tripeptide **2-5** could be formed from a peptide coupling of the suitably protected ϵ -lactam **2-6** and glutamine derivative **2-7** (Scheme 2-2). Lactam **2-6** could, in turn, be created from the alkylation of the commercially available lactam (**2-8**) with methyl bromoacetate (**2-9**). Glutamine derivative **2-7** could result from the amidation of protected glutamine (**2-10**) with benzyl amine (**2-11**).



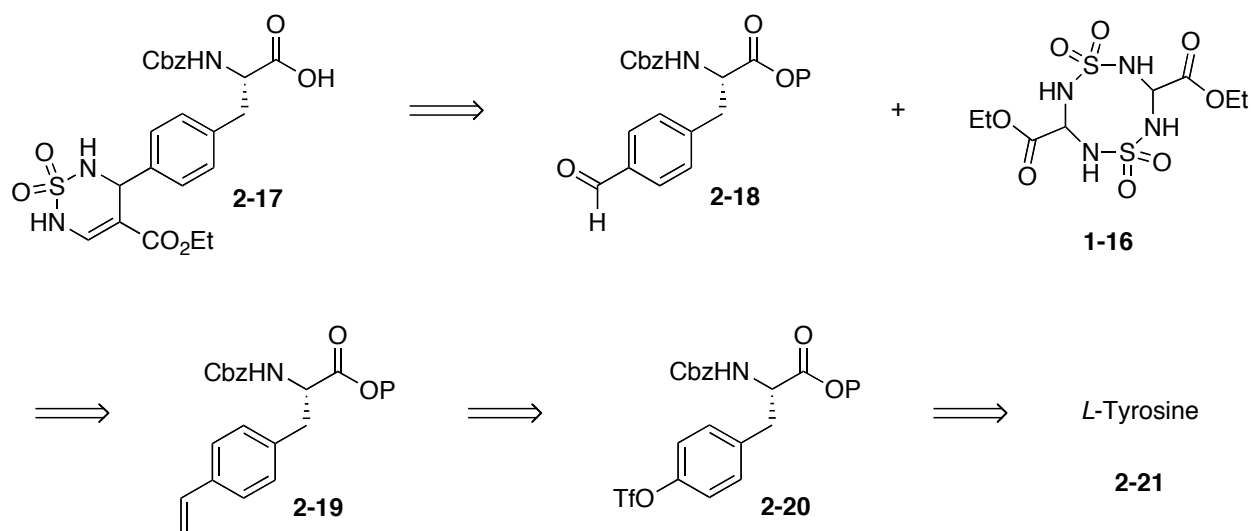
Scheme 2-2. Retrosynthetic analysis of tripeptide **2-5**.

Turning to the tyrosine subunits, IZD-tyrosine derivative **2-12** could be realized by a Suzuki-Miyaura cross-coupling of boronic acid **2-13** and the known IZD coupling partner **2-14** (Scheme 2-3).²² Boronic acid **2-13** could be formed from iodide **2-15**, which would result from iodination of *L*-phenylalanine (**2-16**).



Scheme 2-3. Retrosynthetic analysis of the IZD phosphotyrosine mimic.

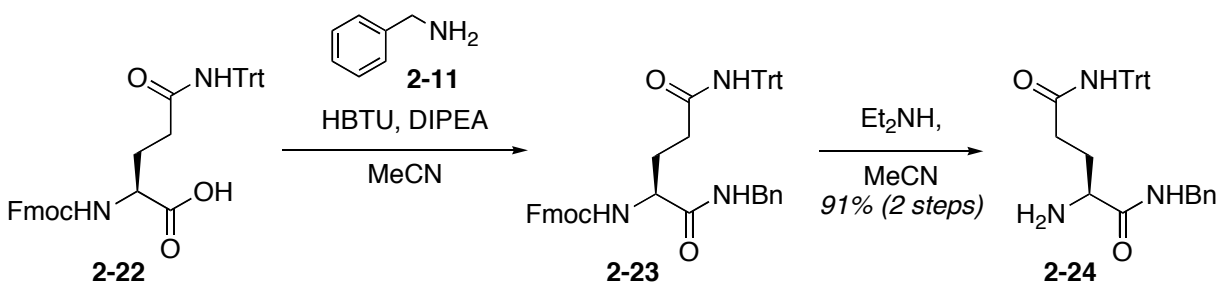
TDA-tyrosine derivative **2-17** could be the result of a condensation of aldehyde **2-18** and the eight-membered heterocycle **1-16** (Scheme 2-4). Aldehyde **2-18** could be formed from the oxidative cleavage of olefin **2-19**. Olefin **2-19** could be formed by a Stille reaction of a vinyl stannane and triflate **2-20**, which could be derived from *L*-tyrosine (**2-21**).



Scheme 2-4. Retrosynthetic analysis of the TDA phosphotyrosine mimic.

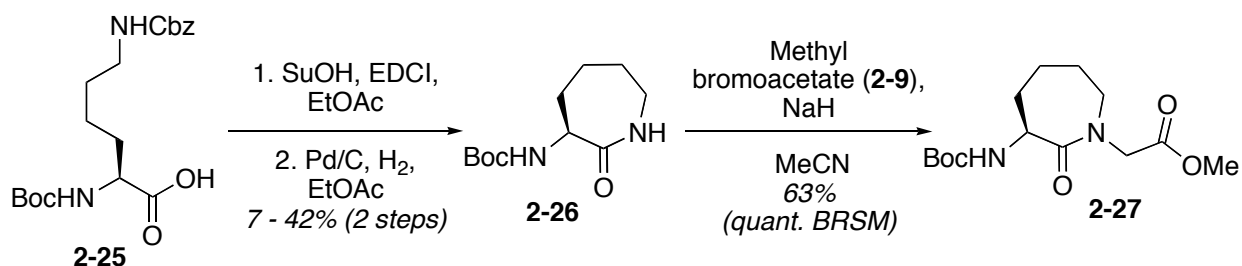
2.3 SYNTHESIS OF FREIDINGER LACTAM FRAGMENT

Commercially available protected glutamate (**2-22**) was coupled with benzyl amine (**2-11**) to form benzyl amide **2-23**, which was used directly in the next step (Scheme 2-5). Treatment with diethylamine afforded free amine **2-24** in 91% over two steps.



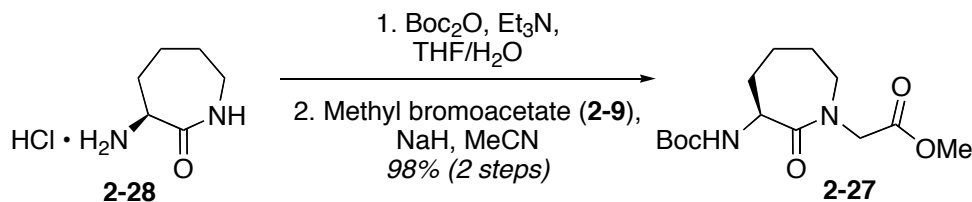
Scheme 2-5. Synthesis of glutamine derivative **2-24**.

Commercially available Boc-Lys(Cbz)-OH (**2-25**) was treated with *N*-hydroxysuccinimide in the presence of EDCI to afford Boc-Lys(Cbz)-OSu (Scheme 2-6). This compound was immediately subjected to hydrogenation with 10% Pd/C in ethyl acetate under a hydrogen atmosphere to deprotect the Cbz group, and the resulting amino acid spontaneously underwent ring closing to form caprolactam **2-26** in variable yields. The amide nitrogen was then alkylated with methyl bromoacetate to afford compound **2-27** in 63% yield (quantitative yield based on recovered starting material).



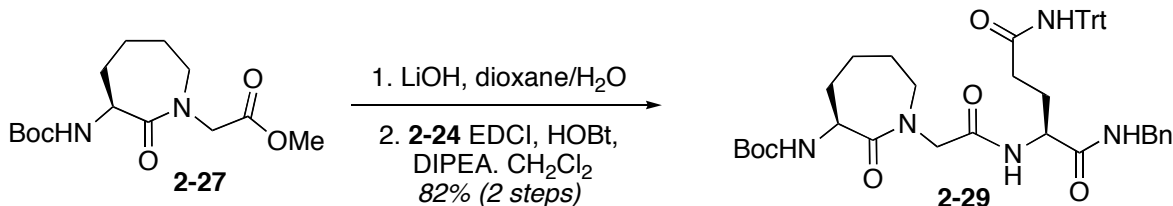
Scheme 2-6. Synthesis of methyl ester **2-27** from bis-protected lysine **2-25**.

Due the poor yields obtained for lactam **2-26**, we switched the starting material to the commercially available *L*-(-)- α -amino-caprolactam hydrochloride (**2-28**) (Scheme 2-7). Boc protection of the amine followed by amide alkylation with methyl bromoacetate (**2-9**) afforded ϵ -lactam **2-27** in 98% yield over two steps



Scheme 2-7. Elaboration of caprolactam **2-28** into methyl ester **2-27**.

With these two coupling partners in hand, it was possible to hydrolyze methyl ester **2-27** and couple it to amine **2-24** with EDCI and HOBt to afford tripeptide **2-29** in 82% yield over two steps (Scheme 2-8).

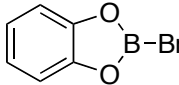


Scheme 2-8. Synthesis of Boc protected tripeptide **2-29**.

Selective deprotection of the Boc protecting group in the presence of the trityl group to arrive at amine **2-30** was unsuccessful (Table 2-1). Treatment with HCl in dioxanes resulted in

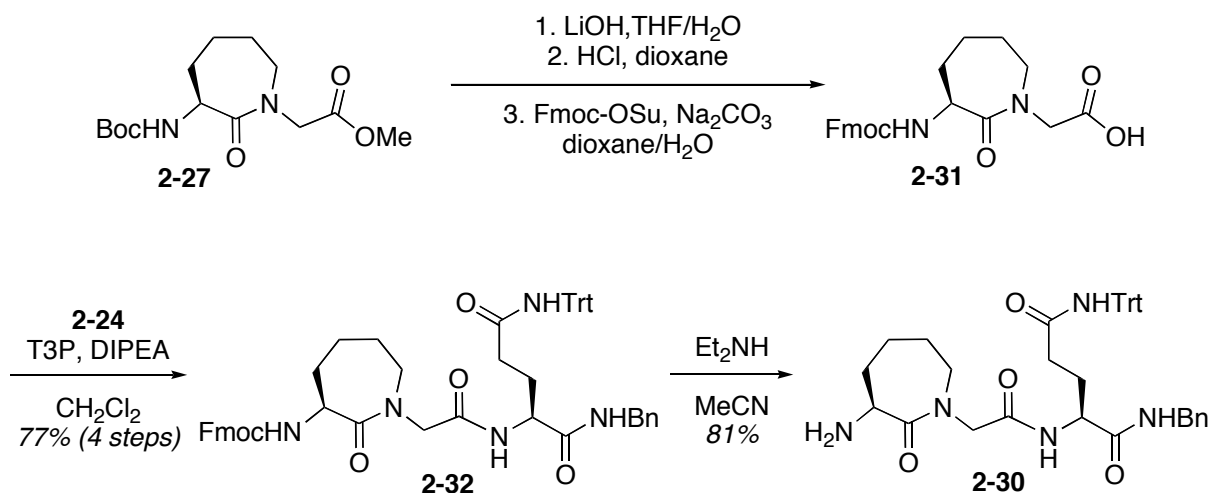
the rapid loss of the trityl group (Table 2-1, entry 1) and buffered TMS triflate resulted in a compound with an incorrect mass by LCMS (Table 2-1, entry 2). Microwave conditions to remove the Boc group resulted in complete recovery of the starting material at both 180 °C and 250 °C (Table 2-1, entries 3 and 4 respectively). An attempt to use *B*-bromocatechol borane resulted in an unidentified compound that was not the desired product (Table 2-1, entry 5).

Table 2-1. Attempts at finding selective Boc deprotection conditions.

entry	conditons	results
1	HCl, dioxane	loss of trityl group
2	TMSOTf, 2,6-lutidine, CH ₂ Cl ₂	incorrect mass by LCMS
3	μW 180°C, <i>o</i> -DCB	no reaction
4	μW 250°C, <i>o</i> -DCB	no reaction
5	 0°C to rt, MeCN	unidentifiable material

Due to the lack of orthogonality between the Boc and trityl group, a protecting group switch was required (Scheme 2-9). Lactam **2-27** was subjected to a three-step sequence of saponification, Boc deprotection, and Fmoc protection to afford Fmoc derivative **2-31**. The crude acid was then coupled to amine **2-24** with T3P¹¹⁹⁻¹²¹ to give tripeptide **2-32** in 77% yield

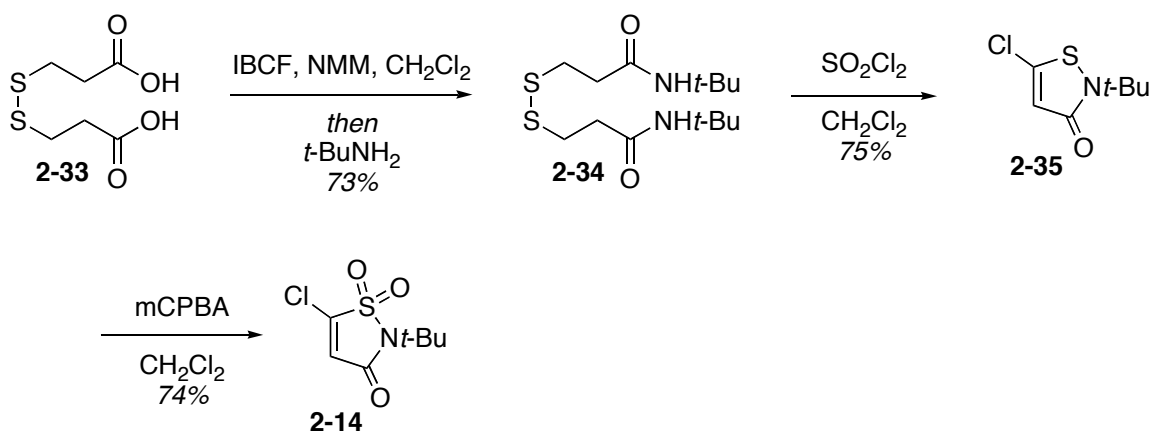
over four steps. Removal of the Fmoc group proceeded smoothly to afford amine **2-30** which provided a compound ready for coupling with the phospho-mimetic tyrosine derivatives.



Scheme 2-9. Protecting group manipulations and construction of tripeptide **2-30**.

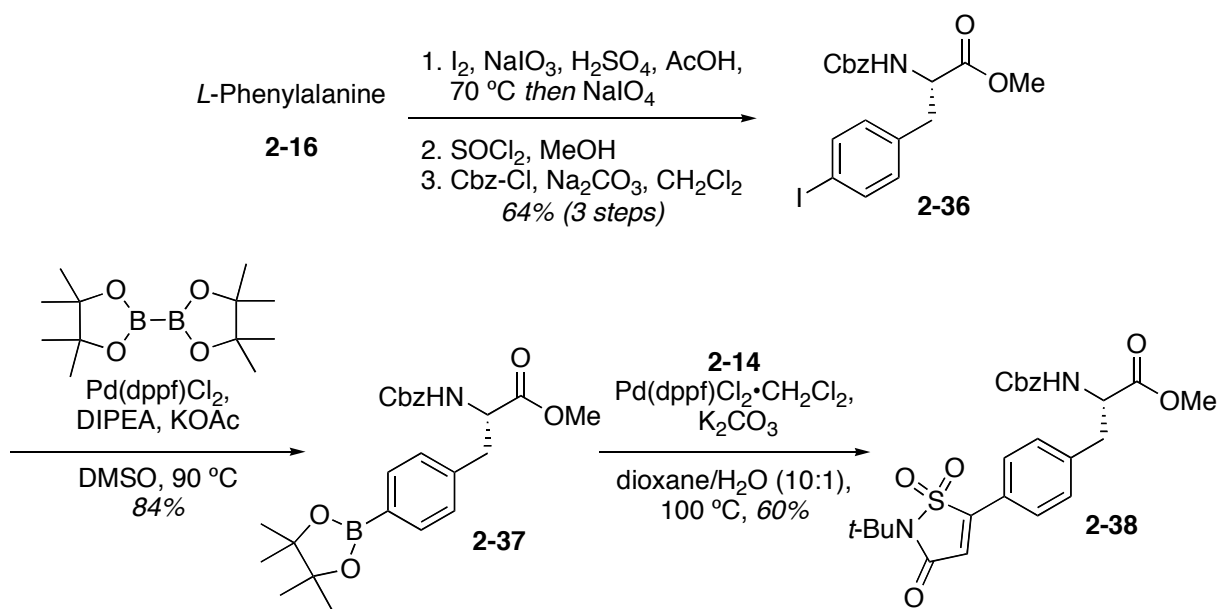
2.4 SYNTHESIS OF THE PHOSPHOTYROSINE DERIVATIVES

Synthesis of the IZD tyrosine derivative began with the synthesis of isothiazolone **2-14** (Scheme 2-10). Treatment of 3,3'-disulfanediylldipropionic acid (**2-33**) with IBCF and *tert*-butyl amine gave diamide **2-34** in 73% yield. Then, following the procedure of Combs et al.,²² the disulfide was treated with sulfuryl chloride at room temperature to give isothiazole **2-35** which was oxidized over three days with mCPBA to give isothiazolone **2-14**.



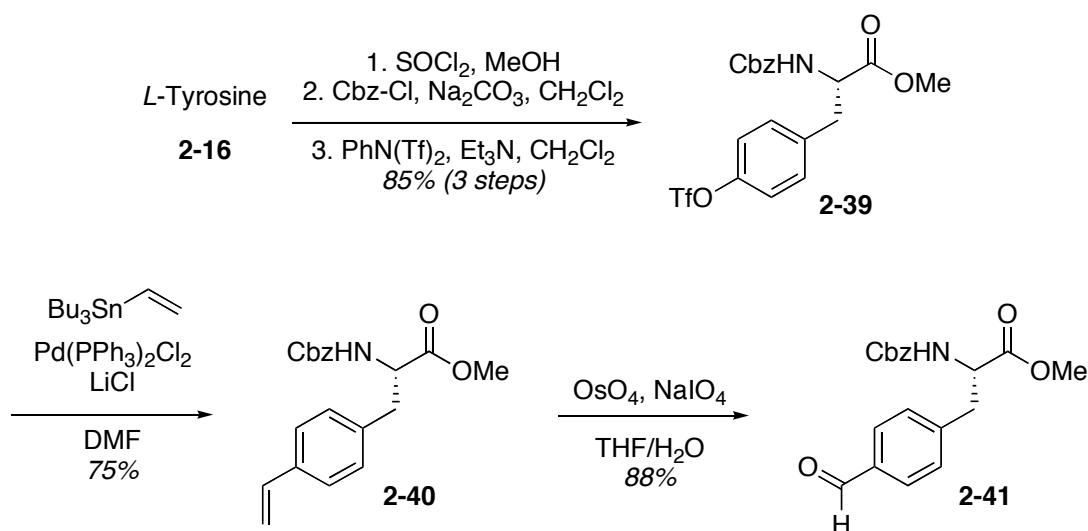
Scheme 2-10. Synthesis of IZD coupling partner **2-14**.

Mono-iodination of *L*-phenylalanine followed by methyl ester formation and Cbz addition gave iodophenylalanine derivative **2-36** in 64% yield over three steps (Scheme 2-11). Palladium catalyzed addition of pinacol borane afforded boronic ester **2-37**, which was further elaborated into IZD phospho-mimetic derivative **2-38** by Suzuki coupling with the isothiazolone **2-14**.



Scheme 2-11. Conversion of phenylalanine (**2-16**) into IZD phosphotyrosine mimetic **2-38**.

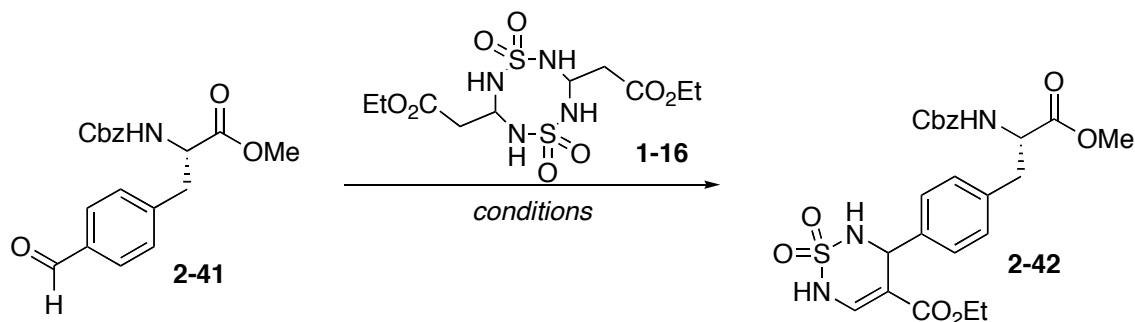
Formation of the TDA derivative began with a three-step process of protection of *L*-tyrosine (**2-16**) as the methyl ester, Cbz addition and triflation of the phenol to provide triflate **2-39** in 85% over three steps (Scheme 2-12). Stille coupling¹²² with tributyl(vinyl)tin afforded olefin **2-40** in 82% yield. Subsequent Johnson-Lemieux oxidative cleavage¹²³ afforded aldehyde **2-41** in 85% yield.



Scheme 2-12. Elaboration of tyrosine into aldehyde **2-41**.

Using the previously optimized conditions for the condensation to the TDA group, treatment of aldehyde **2-41** and dimer **1-16** with 10 equivalents of TFA in CH_2Cl_2 gave only a 15% yield of TDA derivative **2-42** along with the starting material even after 18 h (Table 2-2, entry 1). Raising the amount of dimer **1-16** to three equivalents and changing the solvent to a 1:3 mixture of TFA in CH_2Cl_2 gave a 74% yield after 13 h, but the reaction was very difficult to monitor due to the insoluble nature of dimer **1-16** in the reaction mixture (Table 2-2, entry 2). Lowering the ratio to a single equivalent of dimer **1-16** afforded TDA derivative **2-42** in 83% yield and, as the reaction advanced, the mixture became homogeneous, making it much easier to monitor (Table 2-2, entry 3). Further lowering of the amount of dimer **1-16** to only a half equivalent led to a significant drop in yield to 62% (Table 2-2, entry 4). The reaction was fully optimized with 0.75 equivalents of dimer **1-16** and resulted in the optimal combination of ease of performing the reaction with a good product yield of 83% (Table 2-2, entry 5). These conditions were also amenable to scale up (Table 2-2, entry 6).

Table 2-2. Optimization of TDA phosphotyrosine derivative **2-42**.



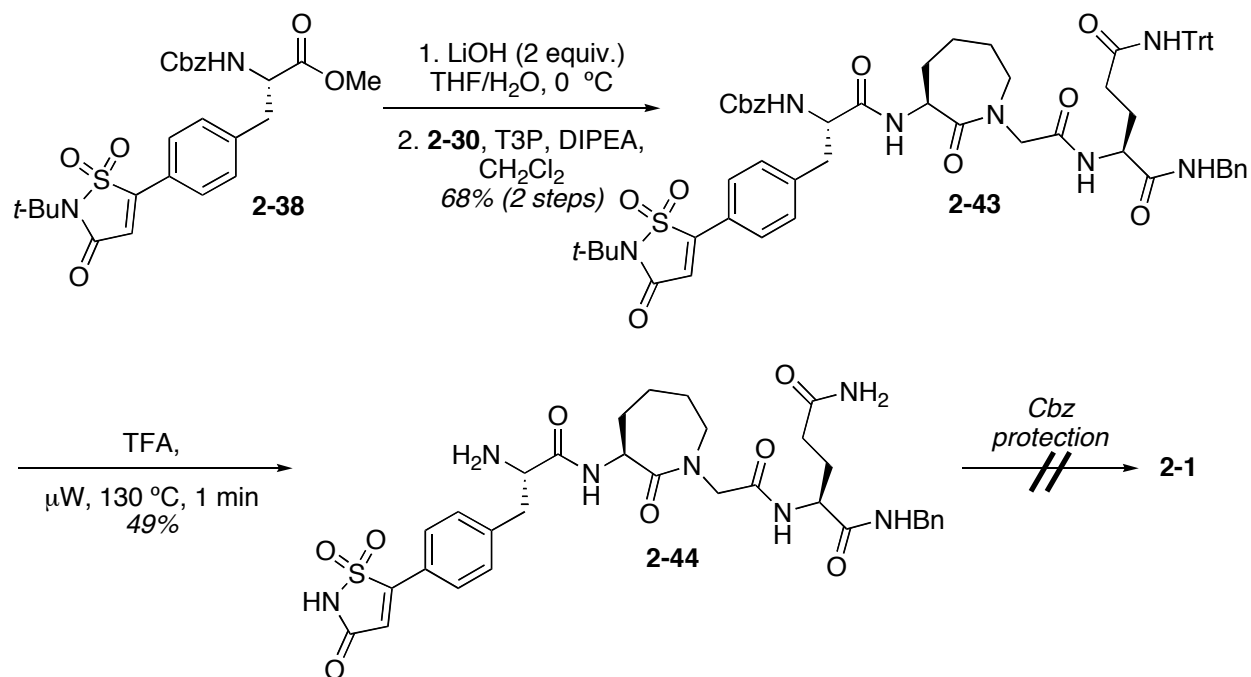
entry	equiv. 1-16	TFA/CH ₂ Cl ₂	time	yield ^a	notes ^b
1	1	10 equiv. TFA	18 h	15%	incomplete rxn
2	3	1:3	13 h	74%	very difficult to monitor
3	1	1:2	5.5 h	83%	heterogeneous rxn mixture
4	0.5	1:2	5.5 h	62%	homogeneous rxn mixture
5	0.75	1:2	5.5 h	83%	50 mg
6	0.75	1.2	5 h	90%	250 mg

^a isolated yield. ^b 40 – 50 mg scale with a concentration of 0.1 M of **2-41** unless otherwise noted.

2.5 FINAL COUPLING AND COMPLETION OF THE SYNTHESIS

After saponification of methyl ester **2-38** with lithium hydroxide at 0 °C, the free acid was coupled with amine **2-30** using T3P to acquire tetrapeptide **2-43** in 68% over two steps (Scheme 2-13). Decomposition of methyl ester **2-38** was observed if excess lithium hydroxide was used or if the temperature was raised to room temperature. Global deprotection removed the trityl, *tert*-butyl, and, unfortunately, the Cbz group (assignment based on proton NMR and mass

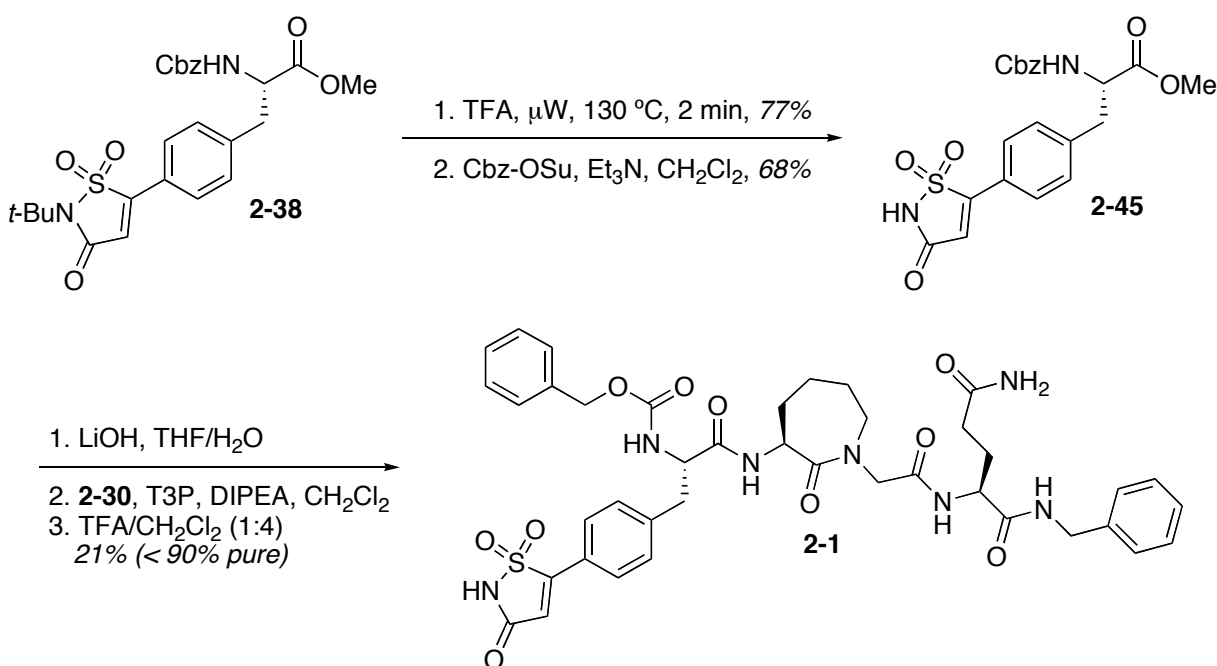
spectrometry). Attempts at reinstalling the Cbz group were unsuccessful due to the limited solubility of the intermediate and the difficulty in analyzing the complex reaction mixture.



Scheme 2-13. Unsuccessful attempt at construction of final target **2-1**.

Returning to IZD phosphotyrosine mimic **2-38**, treatment with TFA in the microwave cleaved both the *tert*-butyl and Cbz groups, and subsequent reprotection with Cbz-OSu and saponification afforded the phosphotyrosine mimic **2-45** (Scheme 2-14). The methyl ester was more robust toward the saponification conditions after removal of the *tert*-butyl group and transformed smoothly to the carboxylic acid. Coupling with tripeptide **2-30** afforded the protected tetrapeptide and treatment with TFA afforded the target tetrapeptide **2-1**. Analysis of the crude reaction mixture by LCMS showed a peak at $R_t = 5$ min. with the desired m/z with a shoulder containing the m/z $[M + 45]$ as the base peak. An additional UV peak was found at $R_t =$

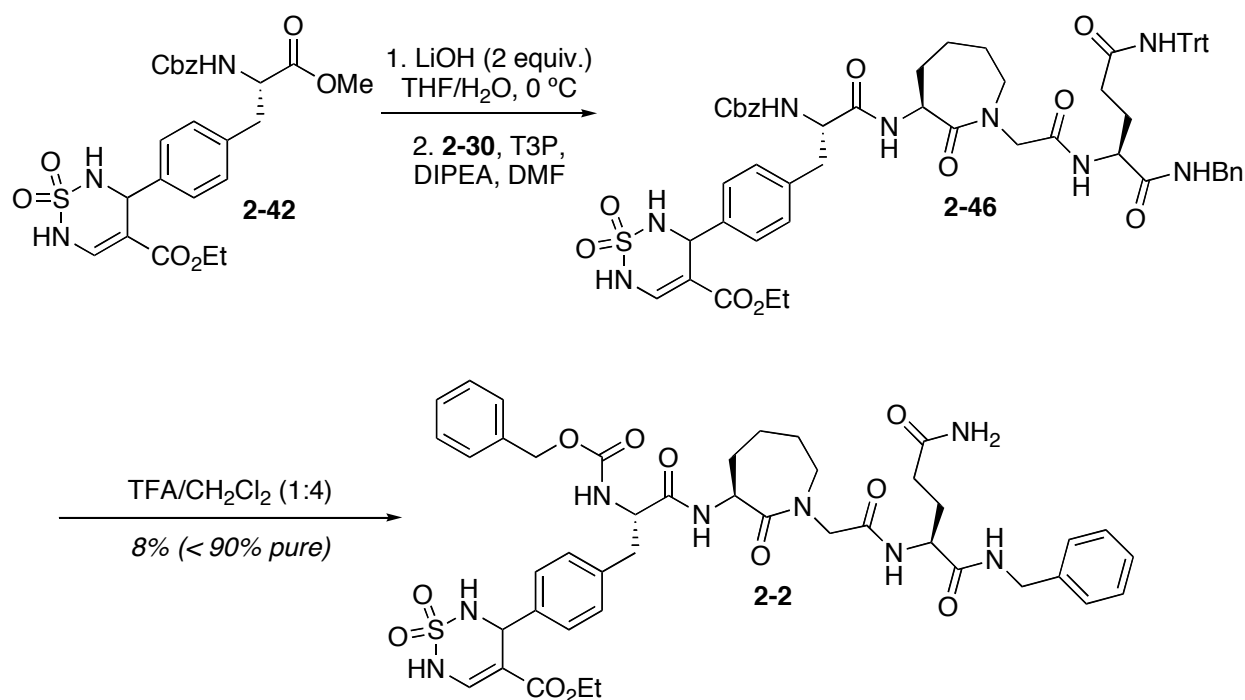
8 min. but no mass ion was obtained. The peak at $R_t = 8$ min. was removed from the mixture by recrystallization from methanol, leaving the compound with its shoulder in the filtrate. Further purification of this compound by recrystallization in methanol/ CH_2Cl_2 , methanol/ethyl acetate, methanol/water, and methanol/isopropanol was unsuccessful. The IZD-tetrapeptide **2-1** was isolated as the major component of a mixture in < 90% purity as determined by ^1H NMR. The nature of the impurities could not be determined.



Scheme 2-14. Synthesis of peptidomimetic **2-1**.

TDA-tyrosine methyl ester **2-38** was saponified with lithium hydroxide at $0\text{ }^\circ\text{C}$ and coupled with amine **2-30** to give compound **2-46** (Scheme 2-15). Removal of the trityl group with TFA afforded the TDA phosphate group mimic containing **2-2**. As with IZD-tetrapeptide **2-1**, TDA analog **2-2** also posed difficulties involved with purification. The crude material was

subjected to HPLC purification and what appeared to be a single peak by LCMS was isolated. This peak contained both the desired m/z 911 $[M + Na]$, and another unknown mass of m/z 943. By comparing the LC/MS spectra with mass ranges limited to $m/z = 910.0 - 912.0$ and m/z 942.0 – 944.0, it was discovered that the two masses corresponded to two separate compounds. Further purification of this compound by recrystallization of methanol/ CH_2Cl_2 , methanol/ethyl acetate, methanol/water, and methanol/isopropanol was unsuccessful. The TDA-tetrapeptide **2-2** was isolated in 8% yield as the major component of a mixture in < 90% purity as determined by 1H NMR.



Scheme 2-15. Synthesis of peptidomimetic **2-2**.

2.6 CONCLUSIONS AND FUTURE DIRECTIONS

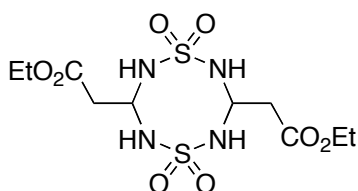
During the first synthetic attempts, a small amount of both tetrapeptides **2-1** and **2-2** were isolated in > 90% purity but needed to be submitted for biological testing before full characterization could be accomplished. Subsequent attempts at re-synthesizing the compounds were when the purity problems arose. Both compounds were found to be inactive in a cellular based assay as peptidic compounds are known to experience trouble traversing the cell membrane. The compounds were also submitted for binding assay studies, and the results are pending.

Future work on this project, pending any activity shown by these samples, should consist of testing both the (*S*)- and (*R*)- derivatives of the hydrogenated IZD phosphotyrosine. Other modifications could include lowering the peptide character of the compounds, including replacement of the amide with hydrogen bond accepting heterocycles such as a triazole. Other β -turn mimics besides the Freidinger lactam could also be tested.

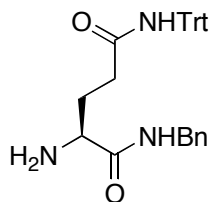
3.0 EXPERIMENTAL SECTION

All reactions were performed under an N₂ atmosphere, and all glassware was flame-dried prior to use. Reactions carried out at -78 °C employed a dry ice/acetone bath. THF was distilled over sodium / benzophenone ketyl. Triethylamine and acetonitrile were distilled from CaH₂, and CH₂Cl₂ and toluene were purified using an alumina filtration system. Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F254 plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), *p*-anisaldehyde solution (2.5 mL of *p*-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H₂SO₄ in 100 mL of 95% EtOH), Vaughn's reagent (4.8 g of (NH₄)₆Mo₇O₂₄·4H₂O and 0.2 g of Ce(SO₄)₂ in 100 mL of a 3.5 M H₂SO₄ solution) or a KMnO₄ solution (1.5 g of KMnO₄ and 1.5 g of K₂CO₃ in 100 mL of a 0.1 % NaOH solution). Flash chromatography on SiO₂ was used to purify the crude mixtures. Microwave reactions were performed in a Biotage Initiator microwave reactor. Melting points were determined on a Nicolet Avatar 360 FT-IR spectrometer. ¹H and ¹³C NMR were obtained on a Bruker Avance 300 instrument. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. ¹H NMR spectra were acquired and tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, dt = doublet of triplet, quint = quintet, m = multiplet, br = broad, app = apparent), number of protons, and coupling constant(s). ¹³C NMR spectra were obtained using a proton-

decoupled pulse sequence with a d1 of 3 sec, and are tabulated by observed peak. LC/MS analyses were obtained from a Hewlett Packard Series 1100 MSD. Mass spectra were obtained on a Micromass Autospec double focusing instrument. Infrared spectra were measured on a Nicolet AVATAR 360 FT-IR E.S.P. spectrometer (KBr or neat) or Smiths Detection IdentifyIR FT-IR spectrometer (ATR).

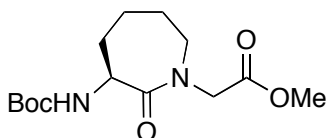


3,7-Dicarbethoxyperhydro-1,2,4,5,6,8-dithiatetrazocine 1,1,5,5-tetraoxide (1-16).^{124,125} A solution of sulfonamide (1.00 g, 10.3 mmol) in CH₂Cl₂/TFA (5:1, 24 mL) was treated with ethyl 3,3-diethoxypropanoate (1.86 mL, 10.3 mmol, 1 equiv) and stirred for 3 h. The mixture was then filtered through a medium glass frit and washed with MeOH to give dimer **1-16** as a white solid (1.67 g, 4.30 mmol, 83%): ¹H NMR (DMSO-d₆, 400 MHz) δ 7.54 (d, 4 H, *J* = 9.2 Hz), 5.180 (tt, 2 H, *J* = 7.2, 9.2 Hz), 4.06 (q, 4 H, *J* = 7.2 Hz), 2.64 (d, 4 H, *J* = 7.2 Hz), 1.18 (t, 6 H, *J* = 7.2 Hz); ¹³C NMR (DMSO-d₆, 100 MHz) δ 168.6, 62.1, 60.2, 41.1, 14.1.



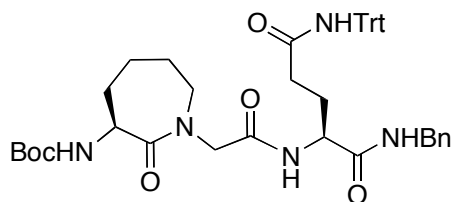
(S)-2-Amino-N¹-benzyl-N⁵-tritylpentanediarnide (2-24).¹¹⁷ A solution of Fmoc-Gln(Trt)-OH (5.00 g, 8.10 mmol), HBTU (3.73 g, 9.73 mmol, 1.2 equiv) and DIPEA (1.5 mL, 8.9 mmol, 1.1 equiv) in MeCN (80 mL) was stirred for 20 min. at room temperature. The

mixture was then treated with benzylamine (0.89 mL, 8.1 mmol, 1 equiv) and stirred overnight. The slurry was then cooled to -10 °C and filtered to give the crude benzyl amide as a white solid (5.16 g, 7.38 mmol, 91%). A slurry of the crude material (1.00 g, 1.43 mmol) in MeCN (20 mL) was treated then with diethylamine (1.5 mL, 14 mmol, 10 equiv) and stirred for 4 h at room temperature. The mixture became clear during the course of the reaction. The solution was condensed, and the resulting residue was purified by chromatography on SiO₂ (0 → 12% methanol in CH₂Cl₂) to give glutamine derivative **2-24** as a white solid (698 mg, 1.46 mmol, 91% over two steps): mp 188.8 – 191.5 °C; $[\alpha]_D^{23} + 2.3$ (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 1644, 1526, 692; ¹H NMR (CDCl₃, 400 MHz) δ 7.49 (br t, 1 H, *J* = 5.2 Hz), 7.27 – 7.15 (m, 20 H), 6.94 (s, 1 H), 4.34 (d, 2 H, *J* = 5.6 Hz), 3.23 (app t, 1 H, *J* = 6.6 Hz), 2.37 (app t, 2 H, *J* = 6.8 Hz), 1.95, 1.85 (app t of AB, 2 H, *J* = 13.8, 6.8 Hz), 1.41 (br s, 2 H); ¹³C NMR (CDCl₃, 100 MHz) δ 174.7, 171.7, 144.6, 138.4, 128.6, 127.9, 127.6, 127.4, 126.9, 70.4, 54.1, 43.0, 33.9, 31.1; HRMS (ESI) *m/z* calcd for C₃₁H₃₂N₃O₂ [M + H] 478.2495, found 478.2472.



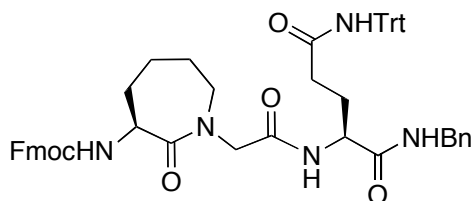
(S)-Methyl 2-(3-(tert-butoxycarbonylamino)-2-oxoazepan-1-yl)acetate (2-27).¹²⁶ A solution of of *L*-(-)-α-amino-caprolactam hydrochloride (1.18 g, 6.95 mmol) and Boc anhydride (1.83 g, 8.34 mmol, 1.2 equiv) in THF/H₂O (3:1, 60 mL) was treated with triethylamine (2.96 mL, 20.9 mmol, 3 equiv) and stirred overnight at room temperature. The reaction mixture was condensed, diluted with water, and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were washed with brine, dried (MgSO₄), filtered, and condensed. A solution of the crude material (assuming 100%) in MeCN (100 mL) at 0 °C was treated with methyl bromoacetate

(1.32 mL, 13.8 mmol, 2 equiv) and NaH (95% in mineral oil, 350 mg, 13.8 mmol, 2 equiv), warmed to room temperature and stirred overnight. The solution was quenched with saturated aqueous NH₄Cl (100 mL), the excess MeCN was evaporated, and the resulting aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried (MgSO₄), filtered, and condensed. The resulting residue was purified by chromatography on SiO₂ (50 → 60% EtOAc in hexanes) to give lactam **2-27** as a light yellow solid (2.04 g, 6.79 mmol, 98% over 2 steps): mp 88.5 – 91.7 °C; [α]_D²³ + 20.3 (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 1748, 1707, 1648; ¹H NMR (CDCl₃, 400 MHz) δ 5.92 (d, 1 H, *J* = 5.6 Hz), 4.43 (dd, 1 H, *J* = 10.8, 6.0 Hz), 4.19, 4.16 (AB, 2 H, *J* = 17.2 Hz), 3.74 (s, 3 H), 3.68 (dd, 1 H, *J* = 15.2, 11.6 Hz), 3.19 (dd, 1 H, *J* = 15.2, 5.2 Hz), 2.08 – 1.97 (m, 2 H), 1.82 – 1.54 (m, 4 H), 1.44 (s, 9 H); ¹³C NMR (CDCl₃, 100 MHz) δ 173.7, 169.6, 155.1, 79.4, 53.4, 52.2, 50.7, 50.6, 32.4, 28.4, 27.9, 27.0; HRMS (ESI) *m/z* calcd for C₁₄H₂₄N₂O₅Na [*M* + Na] 323.1583, found 323.1584.



***tert*-Butyl (*S*)-1-(2-((*S*)-1-(benzylamino)-1,5-dioxo-5-(tritylamino)pentan-2-ylamino)-2-oxoethyl)-2-oxoazepan-3-ylcarbamate (**2-29**).** A solution of methyl ester **2-27** (91.0 mg, 0.302 mmol) in 1,4-dioxane (1.5 mL) was cooled to 0 °C and was treated dropwise with LiOH (2 M in H₂O, 1.06 mL, 2.12 mmol, 7 equiv), warmed to room temperature, and stirred for 40 min. The solution was partitioned between CH₂Cl₂ (5 mL) and water (5 mL). The aqueous layer was acidified to pH < 3, the layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (3 x 5 mL). The organic layer was dried (MgSO₄), filtered, and condensed to give the crude acid

as a white foam which was used in the next step without any further purification. A solution of the crude acid (25.3 mg, 0.0884 mmol), amine **2-24** (69.4 mg, 0.107 mmol, 1.2 equiv), HOBt (130.9 mg, 0.095 mmol, 1.1 equiv), and EDCI (201.2 mg, 0.1048 mmol, 1.2 equiv) in CH₂Cl₂ (0.9 mL) was treated with DIPEA (0.03 mL, 0.2 mmol, 2 equiv) and stirred at room temperature for 20 h. The solution was then diluted with EtOAc (10 mL) and washed with saturated aqueous NH₄Cl (10 mL), NaHCO₃ (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried (MgSO₄) filtered and condensed. The residue was purified by MPLC on SiO₂ (0 → 10% MeOH in CH₂Cl₂) to afford Boc protected tripeptide **2-29** as a white solid (53.9 mg, 0.0723 mmol, 82%, 2 steps): mp 112.0 – 118.9 °C; [α]_D²³ + 2.4 (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 1642, 1508, 1489, 728, 697; ¹H NMR (CDCl₃ + TMS, 400 MHz) δ 7.32 (d, 1 H, *J* = 6.8 Hz), 7.26 – 7.18 (m, 20 H), 7.10 (s, 1 H), 7.06 (br t, 1 H, *J* = 5.2 Hz), 5.80 (d, 1 H, *J* = 5.6 Hz), 4.35 (dd, 1 H, *J* = 13.2, 5.2 Hz), 4.26 (dd, 1 H, *J* = 14.8, 5.2 Hz), 3.91, 3.77 (AB, 2 H, *J* = 15.6 Hz), 3.59 (dd, 1 H, *J* = 14.8, 12.0 Hz), 3.13 (dd, 1 H, *J* = 14.8, 3.6 Hz), 2.51 (app tt, 1 H, *J* = 15.6, 4.4 Hz), 2.35 (app dt, 1 H, *J* = 15.6, 6.0 Hz), 2.07-1.88 (m, 4 H), 1.75-1.62 (m, 2 H), 1.52-1.35 (m, 10 H); ¹³C NMR (CDCl₃, 100 MHz) δ 174.0, 172.2, 170.7, 168.6, 154.9, 144.4, 138.0, 128.6, 128.5, 127.9, 127.6, 127.3, 126.9, 79.3, 70.6, 53.6, 53.3, 52.6, 51.1, 43.4, 33.4, 32.2, 28.7, 28.4, 27.8, 26.8; HRMS (ESI) *m/z* calcd for C₄₄H₅₁N₅O₆Na [M + Na] 768.3739, found 768.3737.

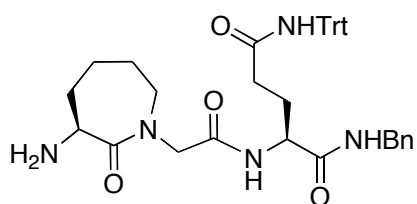


(9H-Fluoren-9-yl)methyl (S)-1-(2-(((S)-1-(benzylamino)-1,5-dioxo-5-(tritylamino)pentan-2-ylamino)-2-oxoethyl)-2-oxoazepan-3-ylcarbamate (2-32).¹¹⁷ A solution of lactam 2-

27 (915 mg, 3.05 mmol) in THF/water (4:1, 20 mL) was treated with 2 N aqueous LiOH (7.6 mL, 15 mmol, 5 equiv) and stirred at room temperature for 2 h. The solvent was reduced under vacuum, diluted with sat. NaHCO₃ (10 mL) and was washed with EtOAc (3 x 30 mL). The aqueous layer was then acidified to pH < 3 and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried (MgSO₄), filtered and condensed. A solution of the crude material (assuming 100%) was stirred in 4 M HCl in dioxane (15 mL) overnight at room temperature. The solvent was removed under vacuum. A solution of the crude material (assuming 100%) in 0.25 M aqueous Na₂CO₃ (36.5 mL, 9.13 mmol, 3 equiv) was treated with Fmoc-OSu (1.18 mg, 3.35 mmol, 1.1 equiv) in dioxane (15 mL) and stirred overnight. The resulting solution was diluted with sat. NaHCO₃ (10 mL) and washed with EtOAc (3 x 30 mL). The aqueous layer was acidified to pH < 3 and extracted with EtOAc (3 x 50 mL). The combined organic layers were then dried (MgSO₄), filtered and condensed to give a white solid that was used without further purification.

A solution of crude carboxylic acid **2-31** (475 mg, 1.16 mmol), glutamine derivative **2-24** (666 mg, 1.39 mmol, 1.2 equiv), and DIPEA (0.38 mL, 2.3 mmol, 2 equiv) in CH₂Cl₂ (5.8 mL) at 0 °C was treated with T3P (50 wt% in EtOAc, 0.81 mL, 1.35 mmol, 1.2 equiv), allowed to warm to room temperature, and stirred for 2 h. The solution was then diluted with EtOAc (25 mL) and washed with NH₄Cl (25 mL), NaHCO₃ (25 mL), and brine (25 mL). The organic layer was dried (MgSO₄), filtered and condensed. The resulting residue was purified by chromatography on SiO₂ (EtOAc) to give Fmoc protected tripeptide **2-32** as a white solid (777 mg, 0.895 mmol, 77%): mp 138.5 – 143.9 °C; [α]_D²³ + 1.4 (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 1644, 1487, 1444, 740, 697; ¹H NMR (CDCl₃ + TMS, 400 MHz) δ 7.75 (d, 2 H, *J* = 10.8 Hz), 7.60 (d, 2 H, *J* = 7.2 Hz), 7.38 (br t, 3 H, *J* = 6.4 Hz), 7.31 – 7.18 (m, 23 H), 7.06 – 7.02 (m, 2 H), 6.08

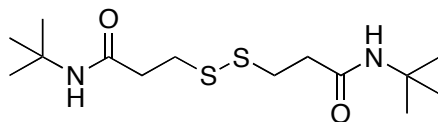
(d, 1 H, $J = 5.6$ Hz), 4.43 – 4.19 (m, 7 H), 3.97, 3.80 (AB, 2 H, $J = 16.0$ Hz), 3.58 (dd, 1 H, $J = 14.8, 12.8$ Hz), 3.13 (dd, 1 H, $J = 15.2, 2.8$ Hz), 2.55 – 2.52 (m, 1 H), 2.40 – 2.36 (m, 1 H), 2.10 – 1.68 (m, 6 H), 1.60 – 1.40 (m, 2 H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 173.8, 172.3, 170.7, 168.5, 155.3, 144.4, 144.0, 143.9, 141.3, 138.0, 128.6, 128.6, 128.0, 127.6, 127.3, 127.0, 127.0, 125.1, 119.9, 70.7, 66.8, 53.8, 53.5, 52.6, 51.0, 47.2, 43.4, 33.5, 32.0, 28.8, 27.7, 26.8; HRMS (ESI) m/z calcd for $\text{C}_{54}\text{H}_{53}\text{N}_5\text{O}_6\text{Na}$ [$\text{M} + \text{Na}$] 890.3894, found 890.3840.



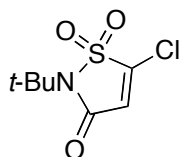
(*S*)-2-(2-((*S*)-3-Amino-2-oxazepan-1-yl)acetamido)-*N*¹-benzyl-*N*⁵-tritylpentane

diamide (2-30).¹¹⁷ A solution of Fmoc protected tripeptide **2-32** (554 mg, 0.638 mmol) in 20% diethylamine in MeCN (8.0 mL) stirred at room temperature for 7 h. During the course of the reaction, a white solid precipitated. The mixture was diluted with CH_2Cl_2 until it became a clear solution and then concentrated under vacuum. The resulting residue was purified by MPLC (silica, 0 → 20 % MeOH in CH_2Cl_2) to give amine **2-30** as a white solid (335 mg, 519 μmol, 81 %): mp 100.0 – 110.0 °C; $[\alpha]_{\text{D}}^{23} + 0.2$ (c 1.0, CH_2Cl_2); FT-IR (ATR) 1644, 1515, 1489, 697; ^1H NMR (500 Hz, $\text{CDCl}_3 + \text{TMS}$) δ 7.64 (d, 1 H, $J = 7.0$ Hz), 7.29 – 7.18 (m, 20 H), 7.10 (br t, 1 H, $J = 5.5$ Hz), 6.96 (s, 1 H), 4.42 – 4.30 (m, 3 H), 4.19, 3.58 (AB, 2 H, $J = 16.0$ Hz), 3.46 – 3.41 (m, 2 H), 3.06 (dd, 1 H, $J = 10.5, 4.5$ Hz), 2.62 (dt, 1 H, $J = 16.5, 6.5$ Hz), 2.44 (dt, 1 H, $J = 16.5, 5.5$ Hz), 2.05 (dd, 2 H, $J = 12.5, 6.5$ Hz), 1.87 – 1.84 (m, 1 H), 1.74 (d, 1 H, $J = 14.0$ Hz), 1.68 – 1.55 (m, 4 H), 1.44 – 1.36 (m, 2 H); ^{13}C NMR (CDCl_3 , 100) δ 177.7, 172.5, 170.9, 169.3, 144.3,

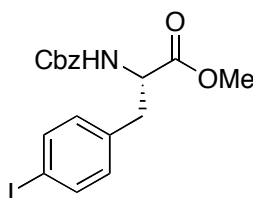
138.2, 128.6, 128.5, 127.9, 127.5, 127.2, 127.0, 70.6, 53.6, 53.5, 52.7, 50.2, 43.2, 33.5, 33.4, 28.0, 27.7, 26.9; HRMS (ESI) m/z calcd for $C_{39}H_{43}N_5O_4Na$ [$M + Na$] 668.3213, found 668.3216.



3,3'-Disulfanediyldis(*N*-*tert*-butylpropanamide) (2-34).¹²⁷ A suspension of 3,3'-dithiodipropionic acid (2.00 g, 9.42 mmol) and NMM (2.50 mL, 22.6 mmol, 2.4 equiv) in CH_2Cl_2 (40 mL) was cooled to $-20\text{ }^{\circ}C$, treated with IBCF (2.95 mL, 22.6 mmol, 2.4 equiv), and stirred at $-20\text{ }^{\circ}C$ for 30 min. The solution was then treated with *tert*-butyl amine (5.10 mL, 47.1 mmol, 5 equiv), warmed to room temperature, and stirred for 2 h. The solution was then quenched with saturated $NaHCO_3$ (150 mL), diluted with EtOAc (150 mL) and separated. The organic layer was washed with saturated NH_4Cl (150 mL), saturated $NaHCO_3$ (150 mL), and brine (150 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by chromatography on SiO_2 (33 \rightarrow 50% EtOAc in hexanes) to give disulfide **2-34** as white crystals (2.19 g, 6.83 mmol, 73%): mp $122.8 - 124.0\text{ }^{\circ}C$; FT-IR (ATR) 3267, 3077, 3963, 2963, 1636, 1556, 1223; 1H NMR ($CDCl_3$, 400 MHz) δ 5.89 (br s, 2 H), 2.91 (t, 4 H, $J = 5.4$ Hz), 2.47 (t, 4 H, $J = 5.4$ Hz), 1.32 (s, 18 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1, 51.2, 36.3, 34.1, 28.7; HRMS (ESI) m/z calcd for $C_{14}H_{28}N_2O_2S_2Na$ [$M + Na$] 343.1490, found 343.1472.

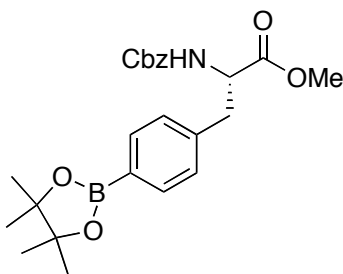


2-tert-Butyl-5-chloro-1,1-dioxo-1,2-dihydro-1 λ^6 -isothiazol-3-one (2-14). Prepared from disulfide **2-34** according to a literature procedure:²² ^1H NMR (CDCl_3 , 400 MHz) δ 6.56 (s, 1 H), 1.69 (s, 9 H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 158.1, 143.7, 123.7, 62.4, 27.7.



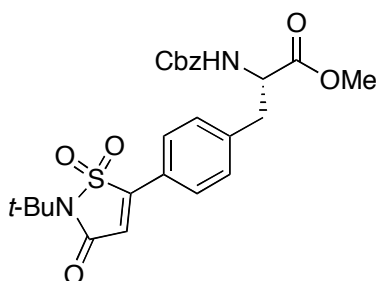
(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-iodophenyl)propanoate (2-36).¹²⁸ A flask was charged with *L*-phenylalanine (**2-16**) (10.0 g, 59.6 mmol), sodium iodate (2.4 g, 12 mmol, 0.2 equiv), iodine (6.1 g, 23.9 mmol, 0.4 equiv), AcOH (53 mL), and H_2SO_4 (7 mL) and warmed to 70 °C for 1.5 d. The solution was treated with NaIO_4 (3 x 650 mg, 2.98 mmol, 0.05 equiv) until the solution changed from dark purple to orange. The solution was then cooled to room temperature and the volatile components were removed under vacuum. The resulting orange oil was diluted to 200 mL with water. The solution was washed with Et_2O (200 mL) and CH_2Cl_2 (200 mL). The aqueous layers were acidified to pH = 5 to give a white sticky precipitate. The solid was immersed in water (1 L) and basified with 10 M NaOH until it was dissolved. The solution was acidified to pH = 5 and the product precipitated out slowly over 2 d. A suspension of the crude iodide (assuming 100%) in MeOH (180 mL, 0.3 M) at 0 °C was treated with SOCl_2 (44 mL, 596 mmol, 10 equiv) dropwise over 30 min. The solution was allowed to warm to room temperature and stirred overnight. The solution was condensed into a light yellow solid. A

suspension of the crude methyl ester (assuming 100%) and Cbz-Cl (9.60 mL, 65.6 mmol, 1.1 equiv) in CH₂Cl₂ (300 mL) was stirred vigorously at 0 °C. The mixture was treated with aqueous Na₂CO₃ (2 M, 33 mL, 65.6 mmol, 1.1 equiv) and stirred at room temperature overnight. The mixture was diluted with CH₂Cl₂ (100 mL) and water (300 mL), the layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2 x 400 mL). The combined organic layers were washed with brine, dried (MgSO₄), and purified by chromatography on SiO₂ (30 → 60% EtOAc in hexanes) to give iodide **2-36** as a white solid (16.76 g, 38.16 mmol, 64% over 3 steps): mp 93.9 – 104.0 °C; [α]_D²³ – 17.7 (*c* 1.0, MeOH); FT-IR (ATR) 1743, 1727, 1689, 1539, 1245; ¹H NMR (CDCl₃) δ 7.59 (dt, 2 H, *J* = 8.0 Hz, 2.0 Hz), 7.40 – 7.32 (m, 5 H), 6.84 (d, 2 H, *J* = 8.0 Hz), 5.25 (d, 1 H, *J* = 8.0 Hz), 5.13, 5.08 (AB, 2 H, *J* = 12.0 Hz), 4.65 (dd, 1 H, *J* = 14.0, 6.0 Hz), 3.73 (s, 3 H), 3.11 (dd, 1 H, *J* = 14.0, 5.6 Hz), 3.01 (dd, 1 H, *J* = 14.0, 6.0 Hz); ¹³C NMR (CDCl₃) δ 171.6, 155.5, 137.6, 136.1, 135.4, 131.2, 128.5, 128.2, 128.1, 92.6, 67.0, 54.5, 52.4, 37.8; HRMS (ESI) *m/z* calcd for C₁₈H₁₈INO₄Na [*M* + Na] 462.0178, found 462.0132.



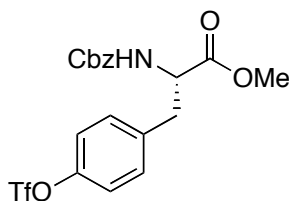
(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (2-37). A solution of iodide **2-36** (559 mg, 1.27 mmol), bis(pinacolato)diboron (359 mg, 1.40 mmol, 1.1 equiv), KOAc (378 mg, 3.81 mmol, 3 equiv) and Pd(dppf)Cl₂ (279 mg, 0.381 mmol, 30 mol%) in DMSO was heated to 80 °C for 2.5 h. The cooled reaction mixture was diluted with water (25 mL) and extracted with EtOAc (4 x 25 mL).

The combined organic layers were washed with brine (3 x 100 mL), dried (MgSO₄), filtered, and concentrated. The resulting residue was purified using chromatography on SiO₂ (30 → 60% EtOAc in hexanes) to give boronic ester **2-37** as a yellow oil (471 mg, 1.07 mmol, 84%): $[\alpha]_D^{23} + 34.7$ (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 2977, 1720, 1357, 1142; ¹H NMR (CDCl₃, 400 MHz) δ 7.74 (d, 2 H, *J* = 8.0 Hz), 7.36 – 7.32 (m, 5 H), 7.12 (d, 2 H, *J* = 8.0 Hz), 5.24 (d, 1 H, *J* = 8.0 Hz), 5.12, 5.08 (AB, 2 H, *J* = 12.4 Hz), 4.68 (dd, 1 H, *J* = 14.0, 6.0 Hz), 3.72 (s, 3 H), 3.16, 3.10 (ABX, 2 H, *J* = 8.0, 6.0 Hz), 1.35 (s, 12 H); ¹³C NMR (CDCl₃, 100 MHz) δ 171.8, 155.5, 138.8, 136.2, 135.0, 132.0, 128.6, 128.5, 128.1, 128.0, 83.7, 66.9, 54.7, 52.3, 38.4, 24.9, 24.8; HRMS (ESI) *m/z* calcd for C₂₄H₃₀BNO₆Na [*M* + Na] 462.2064, found 462.2069.



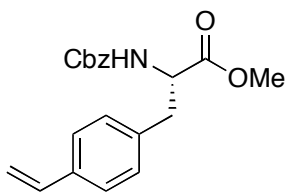
(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-(2-tert-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)phenyl)propanoate (2-38). A solution of boronic ester **2-37** (1.858 g, 4.229 mmol), isothiazolone **2-14** (1.04 g, 4.65 mmol, 1.1 equiv), K₂CO₃ (2.95 g, 21.1 mmol, 5 equiv), and Pd(dppf)Cl₂ (464 mg, 0.634 mmol, 15 mol%) in dioxane/water (10:1, 28 mL) was flushed with Ar for 15 min. and then heated to 100 °C for 3.5 h. The mixture was cooled to room temperature, diluted with EtOAc, and filtered through a cotton plug. The organic layer was washed with water, dried (MgSO₄), filtered and condensed. The resulting residue was purified by chromatography on SiO₂ (10 → 60% EtOAc in hexanes) to give IZD phosphotyrosine derivative **2-38** as a yellow solid (1.27 g, 2.54 mmol, 60%): mp 51.9 °C (dec.); $[\alpha]_D^{23} + 45.2$ (*c* 1.0,

CH₂Cl₂); FT-IR (ATR) 1707, 1510, 1327, 1269, 1152; ¹H NMR (CDCl₃, 400 MHz) δ 7.76 (d, 2 H, *J* = 8.0 Hz), 7.37 – 7.33 (m, 5 H), 7.24 (d, 2 H, *J* = 8.0 Hz), 6.61 (s, 1 H), 5.33 (d, 1 H, *J* = 8.0 Hz), 5.12, 5.08 (AB, 2 H, *J* = 12.4 Hz), 4.70 (dd, 1 H, *J* = 13.6, 6.0 Hz), 3.74 (s, 3 H), 3.24 (dd, 1 H, *J* = 14.0, 5.6 Hz), 3.12 (dd, 1 H, *J* = 14.0, 5.6 Hz), 1.74 (s, 9 H); ¹³C NMR (CDCl₃, 100 MHz) δ 171.4, 160.4, 155.5, 150.2, 141.0, 136.0, 130.4, 128.5, 128.3, 128.1, 128.0, 123.1, 119.1, 67.1, 61.4, 54.5, 52.5, 38.1, 27.7; HRMS (ESI) *m/z* calcd for C₂₅H₂₈N₂O₇SNa [M + Na] 523.1515, found 523.1542.



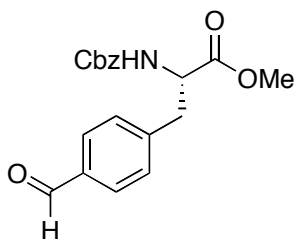
(*S*)-Methyl 2-(benzyloxycarbonylamino)-3-(4-(trifluoromethylsulfonyl)phenyl)propanoate (2-39). A suspension of *L*-tyrosine (**2-16**) (2.00 g, 10.9 mmol) in MeOH (60 mL) at 0 °C was treated dropwise with SOCl₂ (8.0 mL, 109 mmol, 10 equiv) over 5 min and was then heated to reflux for 4 h. The cooled solution was condensed to approx. 1/3 volume and was diluted with Et₂O (250 mL). The white precipitate was filtered off and dried under vacuum overnight. A vigorously stirred suspension of the crude material (assuming 100%) in CH₂Cl₂ (55 mL) at 0 °C was treated with benzyl chloroformate (1.6 mL, 10.9 mmol, 1 equiv) and sodium carbonate (5.5 mL, 2 M in water, 10.9 mmol, 1 equiv). The mixture was warmed to room temperature and stirred for 4 h. The mixture was then diluted with water (50 mL), separated and the aqueous layer was extracted with CH₂Cl₂ (2 x 50 mL). The combined organic layers were washed with brine, dried (MgSO₄), filtered, and concentrated. A solution of the crude material (assuming 100%) and Tf₂NPh (4.1 g, 11.5 mmol, 1.05 equiv) in dry CH₂Cl₂ (55 mL) at 0 °C was

treated with triethylamine (1.7 mL, 12.0 mmol, 1.1 equiv) dropwise over 5 min. The solution was stirred at 0 °C for 1 h, allowed to warm to room temperature, and stirred for 1 h. The solution was then diluted with Et₂O (200 mL) and washed with water (50 mL), 1 M NaOH (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried (MgSO₄), filtered, condensed and purified by chromatography on SiO₂ (20 → 40% EtOAc in hexanes) to give triflate **2-39** as a white solid (4.28 g, 9.27 mmol, 85% over 3 steps): mp 71.3 – 73.2 °C; [α]_D²³ + 36.2 (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 1746, 1690, 1534, 1200, 1135, 882; ¹H NMR (CDCl₃ + TMS, 400 MHz) δ 7.35 – 7.32 (m, 5 H), 7.16 (s, 4 H), 5.39 – 5.31 (m, 1 H), 5.11, 5.06 (AB, 2 H, *J* = 12.0 Hz), 4.65 (dd, 1 H, *J* = 14.0, 6.4 Hz), 3.70 (s, 3 H), 3.18 (dd, 1 H, *J* = 14.0, 5.6 Hz), 3.07 (dd, 1 H, *J* = 14.0, 6.8 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 171.5, 155.5, 148.5, 136.6, 136.0, 131.0, 128.5, 128.2, 128.1, 121.2, 118.6 (q, *J*_{CF} = 310 Hz), 67.0, 54.6, 52.4, 37.5; HRMS (ESI) *m/z* calcd for C₁₉H₁₈F₃NO₇SNa [*M* + Na] 484.0654, found 484.0672.



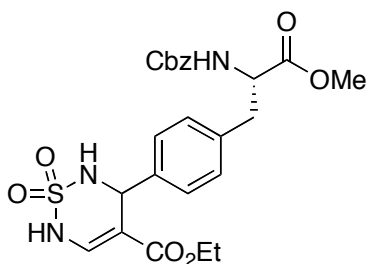
(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-vinylphenyl)propanoate (2-40). A solution of triflate **2-39** (2.02 g, 4.38 mmol), LiCl (558 mg, 13.1 mmol, 3 equiv), and Pd(PPh₃)₂Cl₂ (318 mg, 0.444 mmol, 10 mol%) in degassed DMF (73 mL) was treated with tributyl(vinyl)tin (2.00 mL, 6.57 mmol, 1.5 equiv) and heated to 90 °C for 18 h. The cooled mixture was diluted with Et₂O (200 mL) and washed with saturated aqueous LiCl (3 x 150 mL), 10% aqueous KF (3 x 150 mL) and brine (150 mL). The organic layer was dried (MgSO₄),

filtered and condensed. The resulting residue was purified by chromatography on SiO₂ (20 → 40% EtOAc in hexanes) to give olefin **2-40** as a yellow oil (1.11 g, 3.27 mmol, 75%): $[\alpha]_D^{23} + 39.2$ (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 3029, 2950, 1709, 1510, 1210; ¹H NMR (CDCl₃, 400 MHz) δ 7.39 – 7.32 (m, 7 H), 7.07 (d, 2 H, *J* = 8.0 Hz), 6.70 (dd, 1 H, *J* = 17.6 Hz, 10.8 Hz), 5.74 (d, 1 H, *J* = 17.6 Hz), 5.29 (d, 1 H, *J* = 8.0 Hz), 5.25 (d, 1 H, *J* = 10.8 Hz), 5.13, 5.09 (AB, 2 H, *J* = 12.0 Hz), 4.68 (dd, 1 H, *J* = 14.0, 6.0 Hz), 3.74 (s, 3 H), 3.15 (dd, 1 H, *J* = 8.4 Hz, 5.6 Hz), 3.09 (dd, 1 H, *J* = 8.4 Hz, 6.0 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 171.9, 155.6, 136.4, 136.3, 136.2, 135.2, 129.4, 128.4, 128.1, 128.0, 126.4, 113.7, 66.9, 54.7, 52.3, 37.8; HRMS (ESI) *m/z* calcd for C₂₀H₂₁NO₄Na [*M* + Na] 362.1368, found 362.1398.



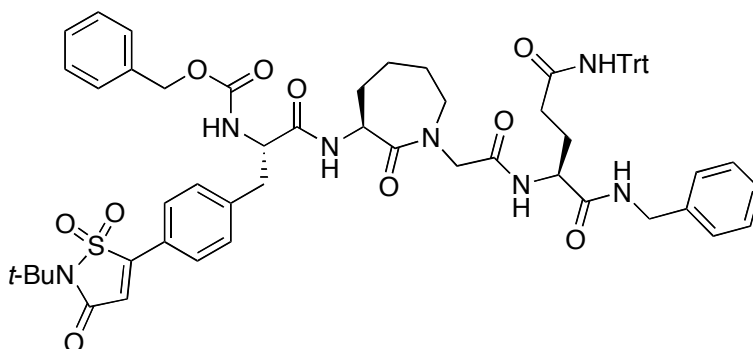
(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-formylphenyl)propanoate (2-41).¹²⁸ A solution of olefin **2-40** (761 mg, 2.24 mmol) in THF/H₂O (3:1, 25 mL) was treated with one drop of 0.1 M OsO₄ in toluene (0.2 mL, 0.02 mmol, 1 mol%) and stirred until the solution was dark brown. The reaction mixture was then treated with NaIO₄ (1.45 mg, 6.73 mmol, 3 equiv), stirred for 2 h at room temperature, diluted with water (40 mL) and extracted with CH₂Cl₂ (3 x 40 mL). The combined organic layers were washed with brine, dried (MgSO₄), filtered and condensed. The resulting residue was purified by MPLC on SiO₂ (21 → 42% EtOAc in hexanes) to give aldehyde **2-41** as a tan solid (674 mg, 1.97 mmol, 88%): mp 67.5 – 69.1 °C; $[\alpha]_D^{23} + 53.9$ (*c* 1.0, CH₂Cl₂); FT-IR (ATR) 1739, 1690; ¹H NMR (CDCl₃ + TMS, 400 MHz) δ 9.97 (s, 1 H), 7.78 (d,

2 H, $J = 8.0$ Hz), 7.37 – 7.31 (m, 5 H), 7.27 (d, 2 H, $J = 8.0$ Hz), 5.32 (br s, 1 H), 5.12, 5.06 (AB, 2 H, $J = 12.0$ Hz), 4.71 (dd, 1 H, $J = 13.6, 6.4$ Hz), 3.73 (s, 3 H), 3.25 (dd, 1 H, $J = 13.6, 5.6$ Hz), 3.13 (dd, 1 H, $J = 13.6, 6.4$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 191.8, 171.5, 155.5, 143.0, 136.0, 135.3, 130.0, 129.9, 128.5, 128.3, 128.1, 67.0, 54.5, 52.5, 38.4; HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{19}\text{NO}_5\text{Na}$ [$M + \text{Na}$] 364.1161, found 364.1171.



(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-(1,1-dioxo-1,2,3,6-tetrahydro-1λ6-[1,2,6]thiadiazine 4-carboxylic acid ethyl ester-3-yl)phenyl)propanoate (2-42). A slurry of aldehyde **2-41** (250 mg, 0.732 mmol) and dimer **1-16** (213 mg, 0.549 mmol, 0.75 equiv) in CH_2Cl_2 (6.0 mL) was treated dropwise with TFA (3.0 mL) and stirred for 4.4 h. The mixture was concentrated and the resulting residue was purified by MPLC on SiO_2 (41 → 62% EtOAc in hexanes) to give thiadiazine **2-42** as a white solid as a mixture of diastereomers (340 mg, 0.657 mmol, 90%): mp 70.1 – 84.6 °C; $[\alpha]_{\text{D}}^{23} + 30.0$ (c 1.0, CH_2Cl_2); FT-IR (ATR) 1692, 1633, 1156; ^1H NMR (CDCl_3 , 400 MHz) δ 7.70 (br s, 1 H), 7.44 (br s, 1 H), 7.40 – 7.30 (m, 5 H), 7.25 (d, 2 H, $J = 8.0$ Hz), 7.10 – 7.07 (m, 2 H), 5.52 (t, 1 H, $J = 7.2$ Hz), 5.37 (t, 1 H, $J = 8.0$ Hz), 5.09, 5.07 (AB, 2 H), 4.90 (2d, 1 H, $J = 8.4$ Hz), 4.65 – 4.63 (m, 1 H), 4.06 – 3.95 (m, 2 H), 3.70 (s, 3 H), 3.14 – 3.04 (m, 2 H), 1.00 (dt, 3 H, $J = 6.4, 6.4$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 172.2, 172.1, 165.5, 155.8, 138.3, 137.0, 136.1, 136.0, 135.9, 135.8, 129.4, 129.4, 128.5, 128.5, 128.2,

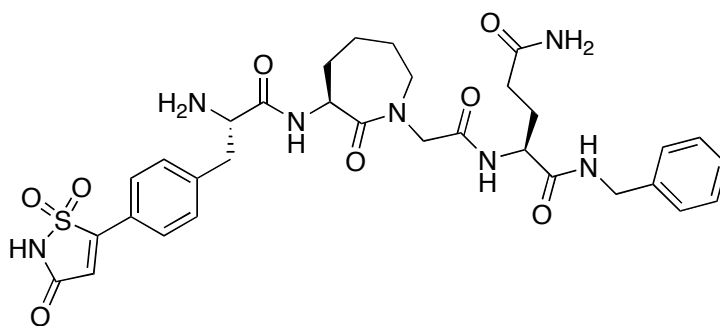
128.1, 127.9, 106.1, 106.0, 67.1, 60.5, 59.1, 59.0, 54.8 (2C), 52.5, 37.9, 37.8, 13.9; HRMS (ESI) m/z calcd for $C_{24}H_{27}N_3O_8SK$ [$M + K$] 556.1156, found 556.1188.



CbzHN-(*tert*-butyl-IZD)Tyr-(2-amido- ϵ -lactam)-Gly-Glu(Trt)-NHBn (2-43). A

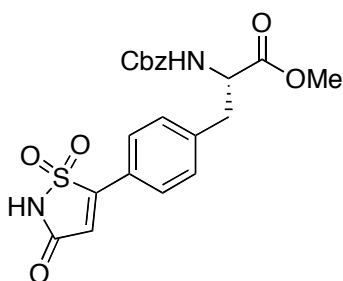
solution of IZD phosphotyrosine derivative **2-38** (103.3 mg, 0.2064 mmol) in THF at 0 °C was treated with 0.2 N LiOH (2.0 mL, 0.4 mmol, 2 equiv) that had been pre-cooled to 0 °C. The solution was stirred for 10 min then quenched with 0.1 N HCl (4 mL), warmed to room temperature and acidified with 1 N HCl (0.5 mL). The mixture was then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and condensed. A solution of the crude material (100 mg, 0.206 mmol), amine **2-30** (159 mg, 248 mmol, 1.2 equiv), and DIPEA (0.07 mL, 0.4 mmol, 2 equiv) in CH_2Cl_2 (2 mL) at 0 °C was treated with T3P (50 wt% in EtOAc, 0.15 mL, 0.25 mmol, 1.2 equiv), allowed to warm to room temperature, and stirred for 2 h. The solution was diluted with EtOAc (10 mL) and washed with saturated aqueous NH_4Cl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL). The organic layer was then dried ($MgSO_4$), filtered and condensed. The resulting residue was purified by chromatography on SiO_2 (EtOAc) to afford tetrapeptide **2-43** as a yellow solid (157 mg, 0.147 mmol, 68% over 2 steps): mp 140.1 – 147.4 °C; FT-IR (ATR) 1709, 1640, 1487, 697; 1H NMR ($CDCl_3$ + TMS, 500 MHz) δ 7.61 (d, 2 H, J = 6.0 Hz), 7.32 – 7.17 (m, 25 H), 7.15 –

7.09 (m, 1 H), 6.96 (br s, 1 H), 6.58 (s, 1 H), 5.50 – 5.46 (m, 1 H), 5.06, 5.01 (AB, 2 H, $J = 9.6$ Hz), 4.53 – 4.51 (m, 2 H), 4.31 – 4.26 (m, 3 H), 3.93 (s, 2 H), 3.57 (t, 1 H, $J = 11.7$ Hz), 3.17 – 3.10 (m, 2 H), 3.02 – 3.00 (m, 1 H), 2.57 – 2.47 (m, 1 H), 2.41 – 2.31 (m, 1 H), 2.10 – 1.75 (m, 6 H), 1.71 (s, 9 H), 1.60 – 1.36 (m, 2 H); ^{13}C NMR ($\text{CDCl}_3 + \text{TMS}$, 100 MHz) δ 173.3, 172.3, 170.8, 169.1, 168.4, 160.5, 155.7, 150.4, 144.4, 141.5, 138.0, 130.6, 128.7, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.5, 127.4, 127.0, 123.0, 119.1, 70.7, 67.1, 61.4, 55.4, 53.3, 52.6, 52.5, 52.5, 51.0, 43.4, 38.6, 38.6, 33.6, 31.4, 29.3, 27.8, 27.6, 26.7; HRMS (ESI) m/z calcd for $\text{C}_{63}\text{H}_{67}\text{N}_7\text{O}_{10}\text{SNa}$ [$\text{M} + \text{Na}$] 1136.4568, found 1136.4562.



$\text{H}_2\text{N}-(\text{H-IZD})\text{Tyr}-(2\text{-amido-}\epsilon\text{-lactam})\text{-Gly-Glu-NHBn}$ (2-44). A solution of IZD-protected tetrapeptide **2-43** (44.9 mg, 0.0403 mmol) in TFA (1.0 mL) was heated in the microwave to 130 °C for 1 min. The reaction mixture was condensed to a brown solid. The solid was dissolved in a minimum amount of methanol and precipitated with CH_2Cl_2 to give fully deprotected tetrapeptide **2-44** as a light red solid (15.8 mg, 0.0199 mmol, 49%, 60% pure by LC/MS): ^1H NMR ($\text{CDCl}_3 + \text{TMS}$, 400 MHz) δ 7.84 (d, 2 H, $J = 8.4$ Hz), 7.44 (d, 2 H, $J = 8.0$ Hz), 7.27 – 7.24 (m, 5 H), 7.22 – 7.19 (m, 1 H), 6.80 (s, 1 H), 4.78 (d, 1 H, $J = 11.2$ Hz), 4.39, 4.34 (AB, 4 H, $J = 14.8$ Hz), 4.26 – 4.19 (m, 2 H), 4.05 (d, 1 H, $J = 16.4$ Hz), 3.77 (dd, 1 H, $J = 15.6, 12.4$ Hz), 3.05 (dd, 1 H, $J = 14.0$ Hz, 8.8 Hz), 2.33 – 2.28 (m, 2 H), 2.16 – 2.07 (m, 1

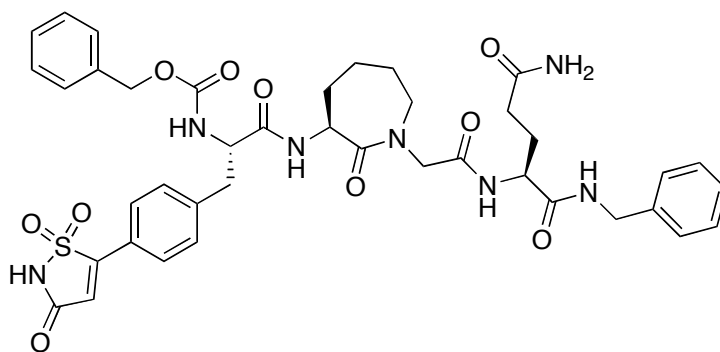
H), 2.00 – 1.90 (m, 3 H), 1.85 – 1.60 (m, 4 H); LC/MS (30 → 100% MeCN in water with 5% MeOH, ESI+) m/z (rel intensity) R_t = 1.29 min.: 704.5 ($[M + Na]^+$, 74), 726.5 ($[M + 2Na - H]^+$, 100);



(S)-Methyl 2-(benzyloxycarbonylamino)-3-(4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)phenyl)propanoate (2-45). A solution of *tert*-butyl IZD tyrosine derivative **2-38** (200.2 mg, 0.4000 mmol) in TFA (2.0 mL) was heated in the microwave for 2 min at 130 °C. The mixture was condensed and purified by reverse phase MPLC (C18, 5 → 20% MeCN in water + 0.1% TFA) to give the free amine as a brown solid (131.1 mg, 0.3089 mmol, 77%).

A solution of the amine (131.1 mg, 0.3058 mmol) in CH_2Cl_2 (3 mL) was cooled to 0 °C and treated with triethylamine (0.17 mL, 1.2 mmol, 4 equiv) and Cbz-OSu (93.3 mg, 0.367 mmol, 1.2 equiv), warmed to room temperature and stirred for 5.5 h. The mixture was condensed and subjected to MPLC purification on SiO_2 (0 → 20% MeOH in CH_2Cl_2) to give the triethylamine salt of **2-45**. The residue was then dissolved in EtOAc (3 mL) and washed with 0.1 N HCl (3 x 3 mL) and brine. The organic layer was dried ($MgSO_4$) filtered and condensed to give IZD phosphotyrosine derivative **2-45** as a tan solid (93.8 mg, 0.211 mmol, 68%): mp 169.8 – 172.9 °C; $[\alpha]_D^{23}$ -27.4 (c 1.0, acetone); FT-IR (ATR) 3349, 2947, 2682, 1715, 1685, 1336, 1157; 1H NMR (acetone- d_6 , 400 MHz) δ 11.23 (br s, 1 H), 7.77 (d, 2 H, J = 8.4 Hz), 7.52 (d, 2

H, $J = 8.4$ Hz), 7.36 – 7.26 (m, 5 H), 7.16 (s, 1 H), 6.77 (d, 1 H, $J = 8.4$ Hz), 5.02 (s, 2 H), 4.55 (dd, 1 H, $J = 9.2, 5.2$ Hz), 3.30 (dd, 1 H, $J = 14.0, 5.2$ Hz), 3.11 (dd, 1 H, $J = 14.0, 9.6$ Hz); ^{13}C NMR (acetone- d_6 , 100 MHz) δ 172.6, 161.7, 156.8, 153.8, 143.7, 138.0, 131.4, 129.2, 129.0, 128.6, 128.5, 124.2, 120.2, 66.8, 56.0, 52.5, 38.0; HRMS (ESI) m/z calcd for compound $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_7\text{SNa}$ [$\text{M} + \text{Na}$] 467.0889, found 467.0865.



CbzHN-(H-IZD)Tyr-(2-amido- ϵ -lactam)-Gly-Glu-NHBn (2-1). A solution of methyl ester **2-45** (49.7 mg, 0.112 mmol) in THF (1.1 mL) was and treated with 0.2 N LiOH (1.7 mL, 0.34 mmol, 3 equiv) and stirred for 1.5 h. The mixture was acidified to pH < 3 using 0.1 N HCl and extracted with EtOAc (3 x 4 mL). The combined organic layers were washed with brine, dried (MgSO_4), filtered and condensed. A solution of the crude acid (assuming 100%), amine **2-30** (86.7 mg, 0.134 mmol, 1.2 equiv), and DIPEA (0.037 mL, 0.22 mmol, 2 equiv) in DMF (1.2 mL) was cooled to 0 °C and treated with T3P (0.080 mL, 0.134 mmol, 1.2 equiv), warmed to room temperature and stirred for 5 h. The solution was then diluted with CH_2Cl_2 (5 mL) and washed with saturated LiCl solution (5 mL), NH_4Cl (5 mL), and NaHCO_3 (5 mL). During the NaHCO_3 wash the solution became a thick emulsion and the mixture was re-acidified and separated. The organic layer was then washed with brine, condensed and filtered. A suspension of the crude protected tetrapeptide (assuming 100%) and triethylsilane (0.021 mL, 0.13 mmol,

1.2 equiv) in CH₂Cl₂ (0.8 mL) was treated with TFA (0.2 mL) and stirred at room temperature for 3 h. The suspension was condensed under a stream of air. An unidentified impurity was recrystallized from the reaction mixture using MeOH (approx. 2 mL) leaving impure tetrapeptide **2-1** in the filtrate. The filtrate was concentrated and attempts at further purification by recrystallization using MeOH/H₂O, MeOH/CH₂Cl₂, MeOH/EtOAc, MeOH/Et₂O, and MeOH/*i*-PrOH were unsuccessful. Tetrapeptide **2-1** was isolated as a white solid contaminated with 0.5 equiv of isopropanol (22.3 mg, 21%, major component of a mixture in < 90% purity by NMR analysis): ¹H NMR (CD₃OD, 400 MHz) δ 8.50 (t, 1 H, *J* = 5.6 Hz), 8.37 (d, 0.6 H, *J* = 7.2 Hz), 8.18 (d, 1 H, *J* = 8.0 Hz), 7.75 (d, 2 H, *J* = 8.0 Hz), 7.35 (d, 2 H, *J* = 8.0 Hz), 7.29 – 7.21 (m, 14 H), 6.69 (s, 1 H), 5.01, 4.95 (AB, 2 H, *J* = 12.4 Hz), 4.74 – 4.66 (m, 1 H), 4.48 – 4.40 (m, 1 H), 4.40 – 4.31 (m, 4 H), 4.24 (d, 1 H, *J* = 16.4 Hz), 4.01 (d, 1 H, *J* = 16.4 Hz), 3.78 – 3.70 (m, 1 H), 3.23 (dd, 1 H, *J* = 13.6, 3.6 Hz), 2.89 (dd, 1 H, *J* = 13.6, 10.0 Hz), 2.29 (t, 2 H, *J* = 6.8 Hz), 2.17 – 2.13 (m, 1 H), 1.96 – 1.87 (m, 4 H), 1.81 – 1.74 (m, 3 H), 1.65 – 1.50 (m, 3 H).

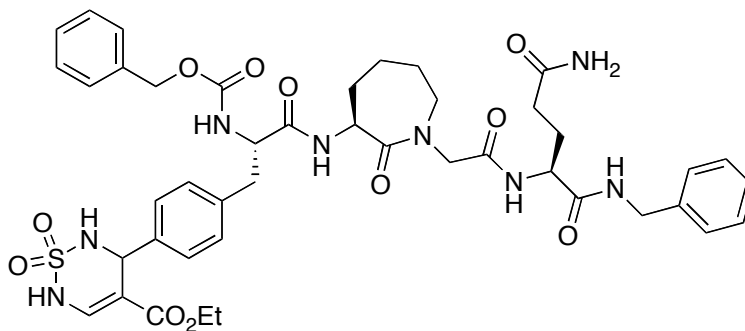
Characteristic peaks of the impurity: 7.47 (d, 0.4 H, *J* = 8.4 Hz), 6.76 (s, 0.15 H); Peaks corresponding to isopropanol: 3.92 (sept., 1 H, *J* = 6.0 Hz), 1.14 (d, 6 H, *J* = 6.0 Hz).

¹³C NMR (CD₃OD, 100 MHz) δ 177.9, 175.3, 173.8, 172.8, 171.4, 158.3, 156.3, 142.2, 139.8, 138.1, 131.1, 129.5, 129.1, 128.6, 128.4, 128.2, 126.5, 123.1, 67.6, 57.5, 54.6, 53.8, 53.7, 51.7, 44.0, 38.8, 32.5, 32.1, 28.7, 28.6, 27.8.

Peaks corresponding to isopropanol: 64.7, 25.2.

LC/MS (30 → 100% MeCN in water with 5% MeOH, ESI+) m/z (rel intensity) R_t = 5.85 min.: 816.4 ($[M + H]^+$, 30), 836.6 ($[M + Na]^+$, 100); Characteristic peak of the impurity: R_t = 5.99 min.: 860 (100).

HRMS (ESI) m/z calcd for $C_{40}H_{45}N_7O_{10}SNa$ $[M + Na]$ 838.2846, found 838.2896.



CbzHN-(TDA)Tyr-(2-amido- ϵ -lactam)-Gly-Glu-NHBn (2-2). A solution of methyl ester **2-42** (49.9 mg, 0.0964 mmol) in THF (1.0 mL) at 0 °C was treated with 0.2 N LiOH (1.0 mL, 2.1 mmol, 2 equiv), warmed to room temperature, and stirred for 1.5 h. The reaction mixture was then acidified with 0.1 N HCl (2.5 mL) and extracted with EtOAc (3 x 4 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and condensed. The residue was isolated as a mixture of diastereomers on the thiadiazine ring and taken on without further purification. A solution of crude the crude acid (assuming 100%), amine **2-30** (74.7 mg, 0.116 mmol, 1.2 equiv) and DIPEA (0.032 mL, 0.19 mmol, 2 equiv) in DMF (1.0 mL) at 0 °C was treated with T3P (0.69 mL, 0.12 mmol, 1.2 equiv), warmed to room temperature, and stirred for 8 h. The reaction was monitored by LCMS. The reaction mixture was concentrated under vacuum and taken on without further purification. A suspension of crude **2-46** (assuming 100%) and triethylsilane (0.021 mL, 0.13 mmol, 1.3 equiv) in CH_2Cl_2 (0.8 mL) was treated with TFA (0.2 mL) and stirred at room temperature for 3 h. The suspension was condensed under a stream

of air. The residue was partially purified by reverse-phase HPLC (30 – 80% acetonitrile in water). Attempts at further purification by recrystallization using methanol/water, methanol/CH₂Cl₂, methanol/ethyl acetate, methanol/ethyl ether, and methanol/isopropanol were unsuccessful. Tetrapeptide **2-2** was isolated as a white solid contaminated with 0.2 equiv of isopropanol (12.0 mg, 8%, major component of a mixture in < 90% purity by NMR analysis): FT-IR (ATR) 3062, 3030, 2950, 1727, 1689, 1539, 1256; ¹H NMR (CD₃OD, 400 MHz) δ 7.58 (d, 1 H, *J* = 2.0 Hz), 7.33 – 7.15 (m, 14 H), 5.42 (s, 1 H), 5.01 – 4.95 (m, 2 H), 4.71 (d, 1 H, *J* = 11.2 Hz), 4.41 – 4.30 (m, 4 H), 4.22 (d, 1 H, *J* = 16.4 Hz), 4.03 – 3.90 (m, 2 H) (partially obscured by isopropanol), 3.76 (dd, 1 H, *J* = 14.8, 12.0 Hz), 3.13 (dd, 1 H, *J* = 13.6, 4.0 Hz), 2.90 – 2.80 (m, 1 H), 2.31 – 2.20 (m, 2 H), 2.17 – 2.06 (m, 1 H), 2.00 – 1.68 (m, 5 H), 1.65 – 1.50 (m, 2 H), 1.02 (t, 1.5 H, *J* = 7.2 Hz), 1.01 (t, 1.5 H, *J* = 7.2 Hz).

Characteristic peaks of the impurity: 5.42 (s, 0.7 H), 2.64 (s, 0.7 H), 0.89 – 0.84 (m, 0.6 H); Peaks corresponding to isopropanol: 3.92 (sept., 1 H, *J* = 6.0 Hz), 1.14 (d, 6 H, *J* = 6.0 Hz).

¹³C NMR (CD₃OD, 100 MHz) δ 177.9, 175.3, 173.7, 173.1, 171.4, 167.9, 158.3, 141.8, 139.8, 139.1, 138.0, 130.1, 129.5, 129.5, 129.2, 129.0, 128.7, 128.4, 128.2, 67.7, 61.2, 59.5, 57.8, 54.6, 53.8, 53.6, 51.7, 44.0, 40.4, 38.4, 32.5, 32.2, 28.8, 28.6, 27.8, 14.5.

Peaks corresponding to isopropanol: 64.7, 25.2.

LC/MS (30 → 100% MeCN in water with 5% MeOH, ESI+) *m/z* (rel intensity) *R*_t = 6.11 min.: 889.4 ([M + H]⁺, 30), 911.5 ([M + Na]⁺, 100); Characteristic peaks of the impurity: *R*_t = 6.38 min.: 943 (100)

HRMS (ESI) *m/z* calcd for C₄₃H₅₂N₈O₁₁SNa [M + Na] 911.3374, found 911.3351.

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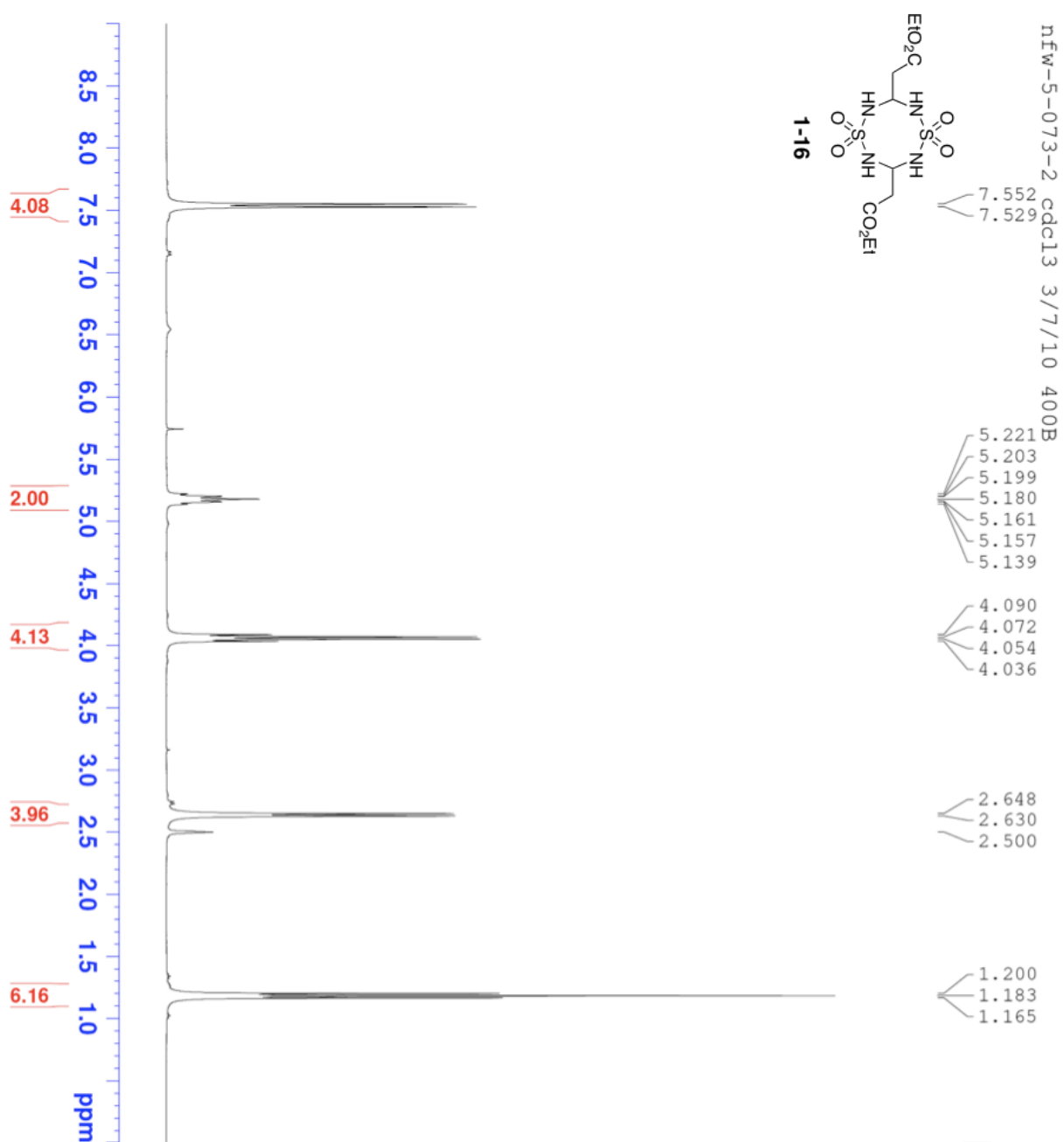
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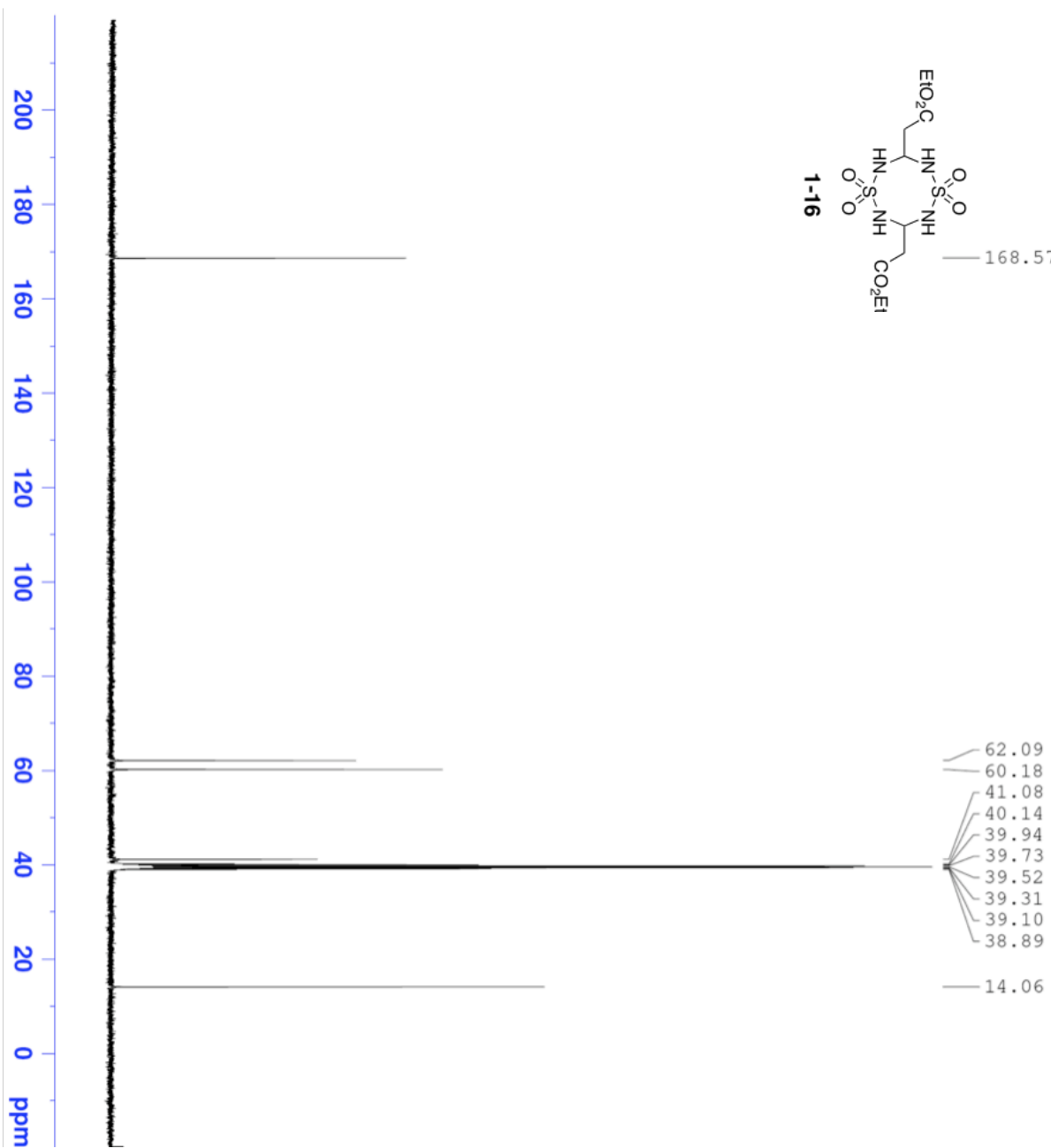
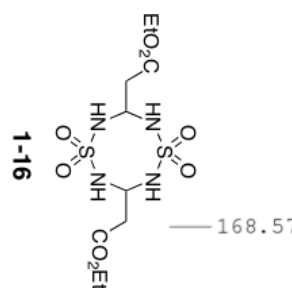
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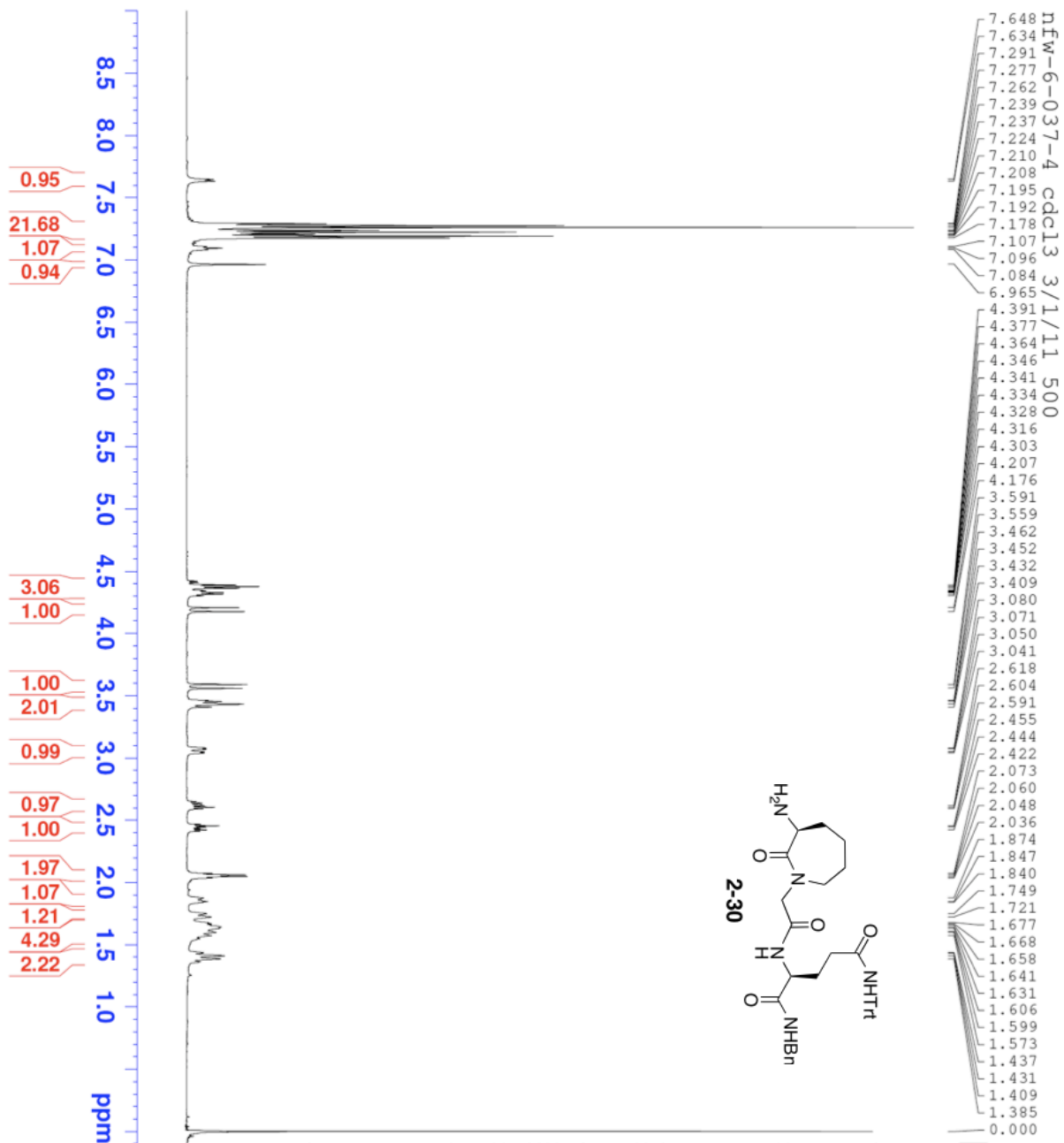
APPENDIX A

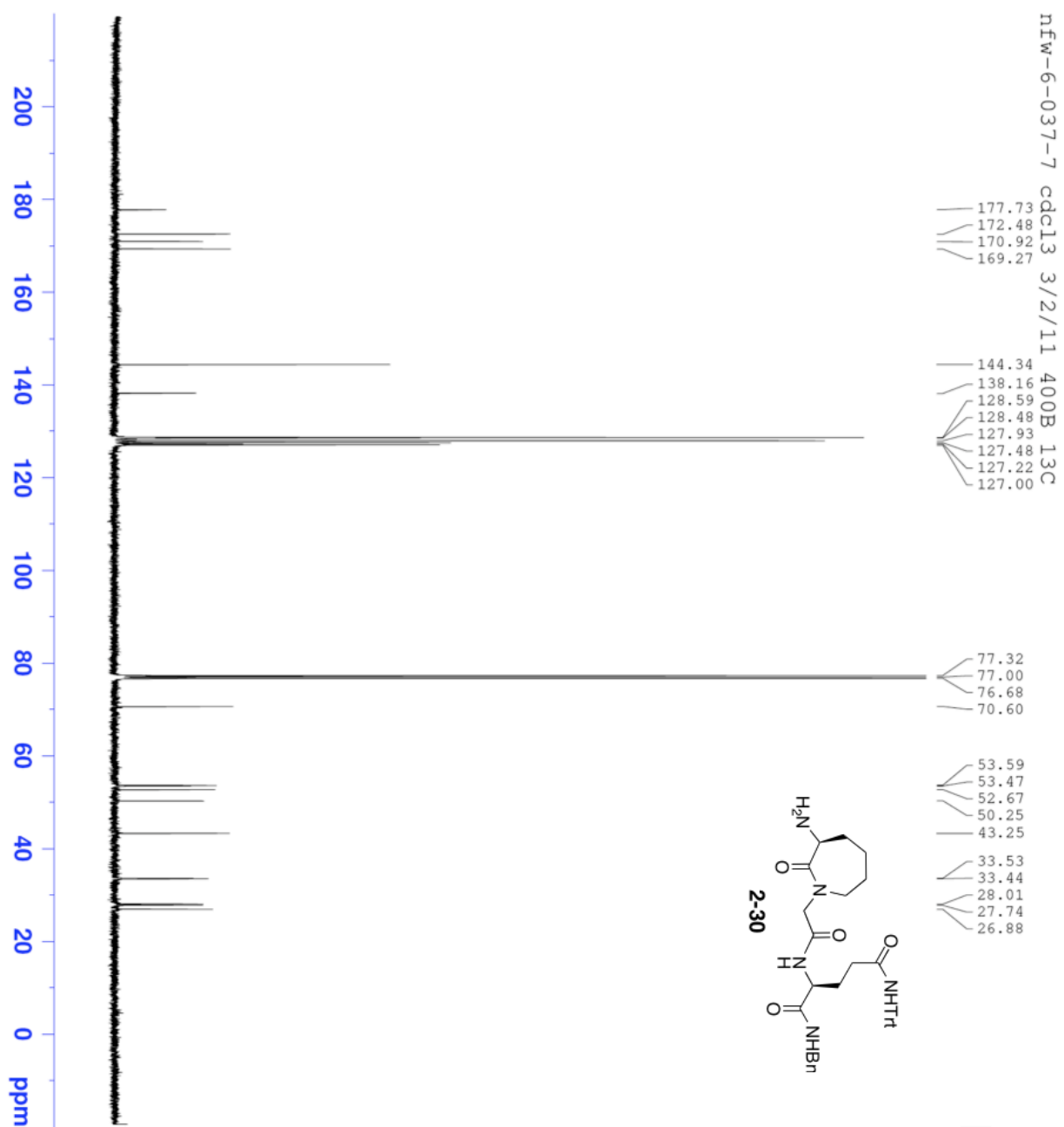
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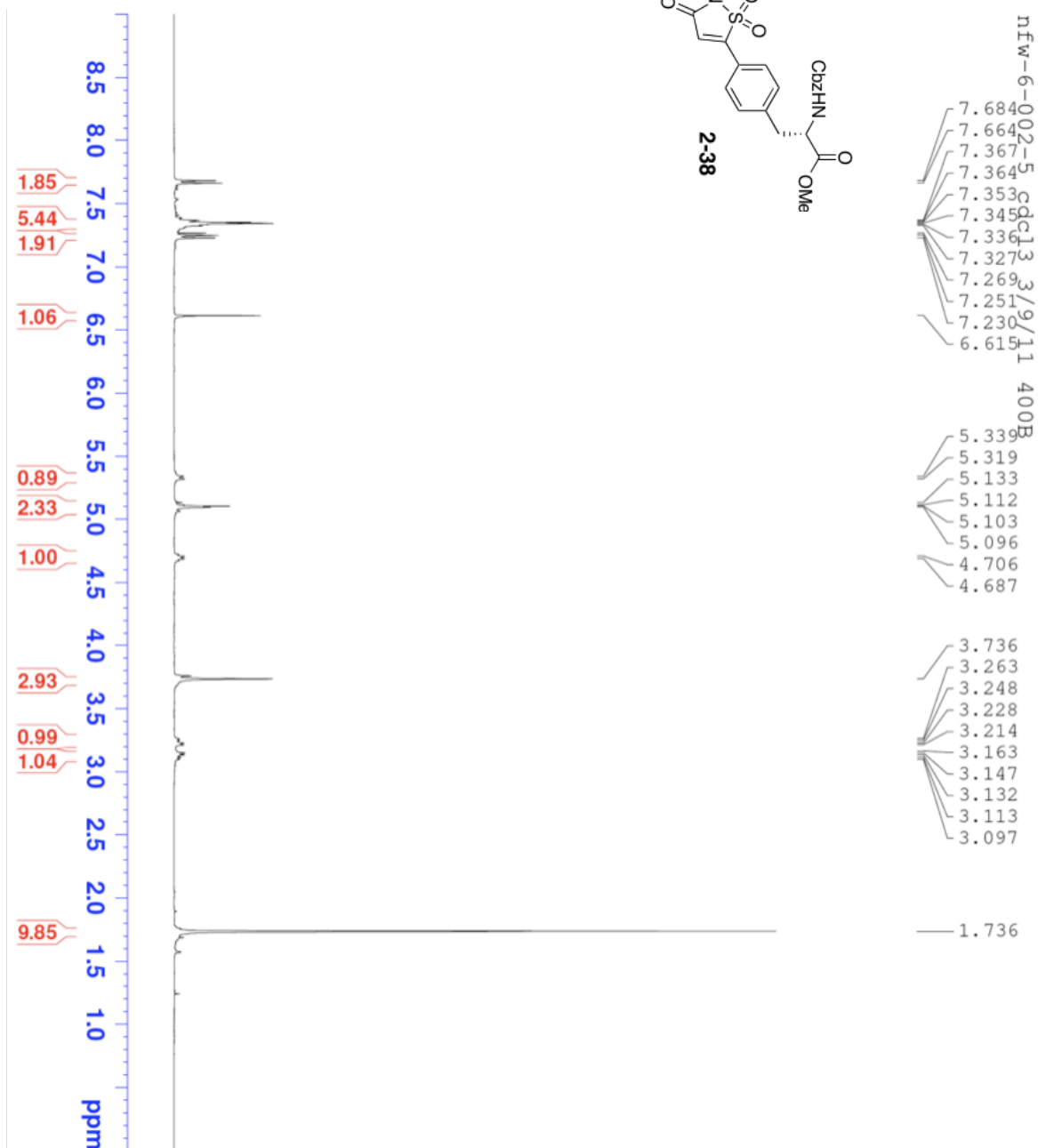
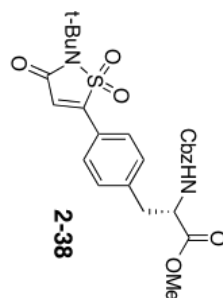


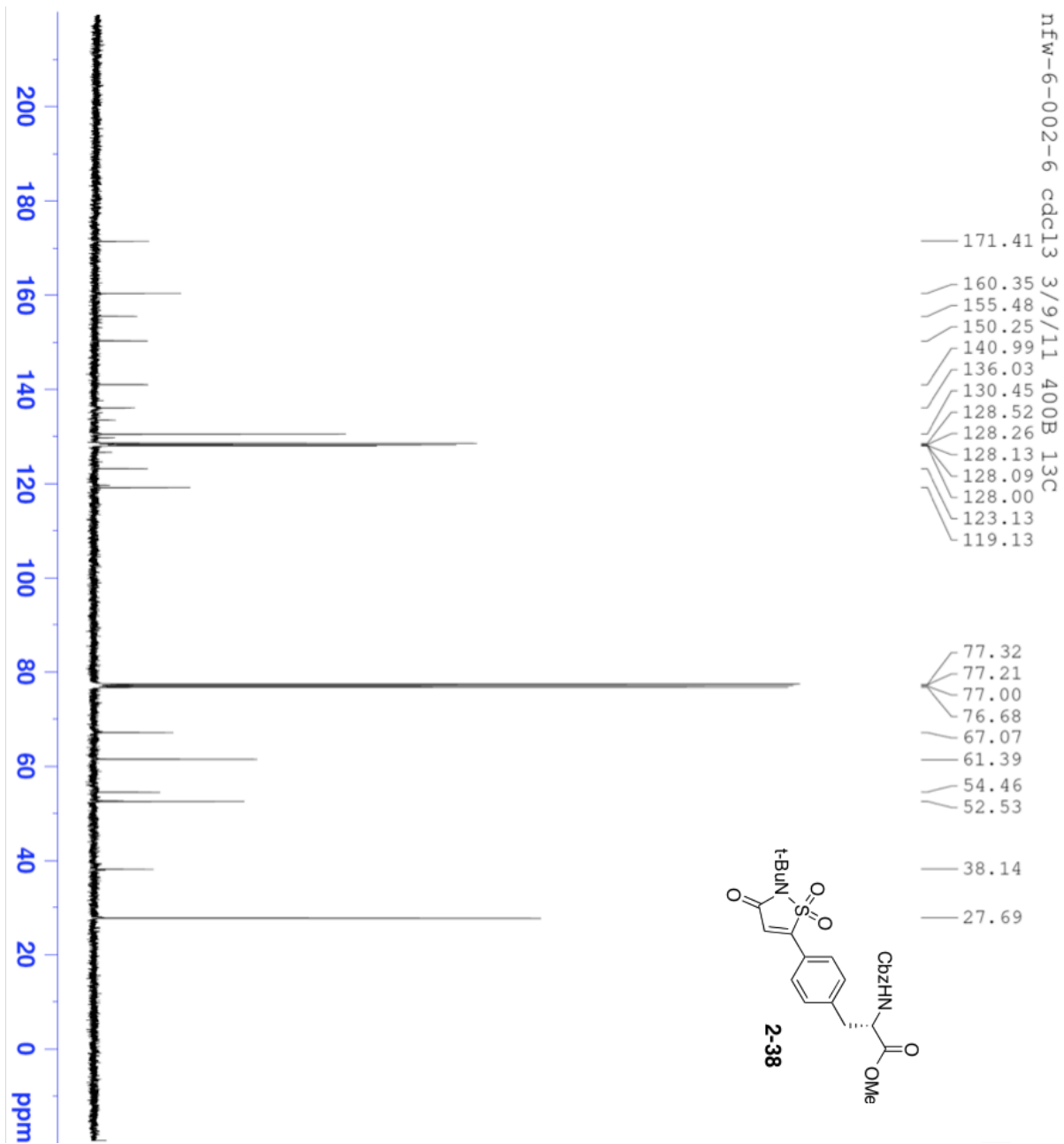
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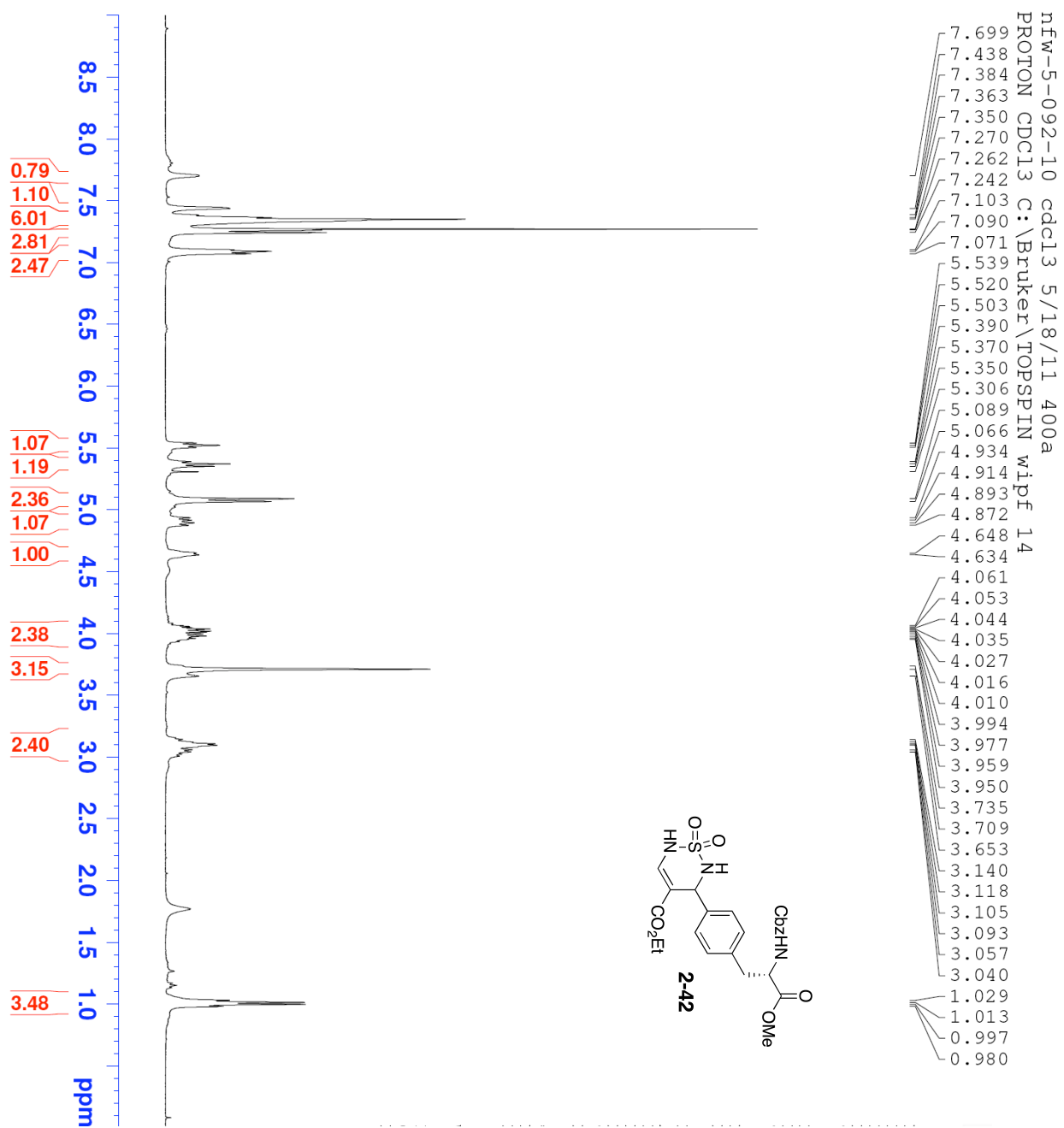












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